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PSYLLID YELLOWS OF THE POTATO[1]

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INTRODUCTION

In August, 1927, Richards, Blood, and Linford [3] reported for the first time what appeared to be a new disease of the potato. Although it resembled current-year symptoms of witches' broom, as described by Hungerford and Dana (*8*),[4] subsequent studies clearly differentiated between the two disorders. In December, 1927, Richards (*14*) ascribed this new disease to the feeding process of the tomato psyllid (*Paratrioza cockerelli* Sulc). He further reported the disease, as it appeared in Utah, as new to science and proposed the name "yellows."[5]

Subsequent statements have been made concerning the occurrence and the destructive nature of the disease by Linford,[6] Richards (*15*),[7] Metzger and Binkley,[8] and Binkley (*1*). Binkley (*1*) reported the disease, as it appears in the tomato, to be due to a definite virus transmitted by the psyllid nymphs. To the writers' knowledge no further report as to the nature of the disease and its relation to the tomato psyllid has appeared. It is the purpose of this paper to bring together the facts known to date regarding psyllid yellows and to report some additional experimental findings regarding the nature and cause of the trouble. The information regarding the disease is recognized definitely as being incomplete.

OCCURRENCE OF PSYLLID YELLOWS

Psyllid yellows has scarcely a parallel in the history of phytopathology in the uniformity and rate with which it spread. The disease was first noticed by the junior author on June 12, 1927, in experimental plots at Farmington, Utah. In a preliminary survey on June 15

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[2] The writers wish to express appreciation for the helpful cooperation of Kathleen L. Hull, formerly of the station staff, and of William Stuart and Melvin Anderson, research students in the Department of Botany and Plant Pathology.

[3] RICHARDS, B. L., BLOOD, H. L., and LINFORD, M. B. DESTRUCTIVE OUTBREAK OF UNKNOWN POTATO DISEASES IN UTAH. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 11:93-94. 1927. [Mimeographed.]

[4] Reference is made by number (italic) to Literature Cited, p. 216.

[5] Since the early proposal of "yellows," the name "psyllid yellows" has been employed generally by the authors and their coworkers in both correspondence and in general discussions and by the senior author in recent publications (*14, 15*). Binkley in 1930 also used the term "psyllid yellows" to designate the disease on tomatoes (*1, Proc. 26, pp. 249-254*). The name "psyllid yellows" seems justifiable as it is both specific and descriptive.

[6] LINFORD, M. B. FURTHER OBSERVATIONS ON UNKNOWN POTATO DISEASE IN UTAH. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 11:110-111. 1927. [Mimeographed.]

——— PLANT DISEASES IN UTAH IN 1927. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Rptr. Sup. 59:95-99. 1928. [Mimeographed.]

[7] RICHARDS, B. L. PSYLLID YELLOWS (CAUSE UNDETERMINED). U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 68:28-29. 1929. [Mimeographed.]

[8] METZGER, C. H., and BINKLEY, A. M. PSYLLID YELLOWS (CAUSE UNDETERMINED). U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 68:29. 1929. [Mimeographed.]

and 16, the disease was found generally distributed in early potato fields of the Bountiful district of Davis County. Most of the fields at this early date showed 100 per cent infection, and not a single field was found which did not show the trouble to some degree. By June 29, at which time Linford commenced a more detailed survey of the disease, the early potatoes in Davis and Weber Counties were generally infested. Before the survey was concluded on September 15, Linford reported that the "disease had been found in every county of Utah where potatoes had been examined, ranging from Washington County on the south to Cache County on the north."

Table 1, copied from Linford's report,[9] shows the occurrence of the disease in 23 counties visited. No reports were obtained from the other six counties.

TABLE 1.—*Occurrence of psyllid yellows in Utah, by counties, 1927* [a]

County	Fields examined	Fields infested		Heaviest infestation	Average infestation
	Number	*Number*	*Per cent*	*Per cent*	*Per cent*
Cache	16	9	56.2	100	53.1
Carbon	16	15	100	100	61.1
Beaver	5	5	100		84
Boxelder	5	5	100	100	53.2
Davis	32	32	100	100	56.6
Duchesne	5	5	100	100	91
Emery [b]					
Garfield	4	4	100	100	61.2
Grand	1	1	100	100	100
Iron	21	21	100	100	83.8
Juab	1	1	100	10	10
Kane	4	4	100	100	70
Millard	17	9	52.9	97	24.2
Morgan [c]					
Piute	11	11	100	100	38.2
Salt Lake	24	13	54.2	100	21.7
Sanpete	12	12	100	100	95.8
Sevier	10	10	100	100	80.8
Uinta	6	6	100	100	68
Utah	9	9	100	100	44
Weber	11	11	100	100	74.9
Wasatch [d]					
Washington [e]					

[a] The figures in this table include data from many fields examined before the disease had reached its maximum spread. From the following publication: LINFORD, M. B. Op. cit., p. 98.
[b] Disease said to be present throughout.
[c] Disease reported prevalent late in season.
[d] Disease present.
[e] Crop a total failure from this disease.

Early in 1927 the malady was reported from the western slope of Colorado and was severe especially in the Fruita district of Mesa County, where 100 per cent infection occurred. According to Metzger and Binkley,[10] the ailment was found also in the Gunnison Valley in Delta County, in the Uncompahgre district in Montrose County, and in the Rifle district of Garfield County.

On August 1, the senior author located psyllid yellows in southern Idaho, 3.5 miles north of Pocatello. Two weeks later M. B. McKay reported the disease by letter from Idaho Falls and Twin Falls, Idaho, and from Bozeman, Mont., and H. G. McMillan in personal conference reported it from Wyoming. L. F. Nuffer by correspondence later in the summer reported a general spread of the disease in the

[9] LINFORD, M. B. Op. cit. See footnote 6, second reference.
[10] METZGER, C. H., and BINKLEY, A. M. Op. cit.

Idaho Falls district. Shapovalov [11] has also called attention to its presence in California.

Although the tomato psyllid (*Paratrioza cockerelli*) has been known since 1914 to be generally distributed in the Southwestern States, the writers have been unable to find any mention in literature, prior to 1927, of a disease of the potato caused by the insect; neither has reference been found to a disease of the potato similar to psyllid yellows. Comperé (*2, p. 189*), in 1915, states: "In Golden Gate Park, San Francisco, the solanums that were infested with psyllid were rendered worthless." He makes no further reference to the disease phase.

It appears evident from a number of authentic reports that the disease occurred previous to 1927. In 1929 Metzger and Binkley [12] stated:

> The disease was destructive in the Fruita district of Mesa County, Colo., in 1926, and by June 18, 33 per cent of the plants were showing symptoms of a disease, evidently psyllid yellows as described by Richards in 1927.

It is quite possible also that the disease was present in the Fruita district prior to 1926. Linford [13] submits data which show rather conclusively that psyllid yellows was a factor in potato production in the Green River district of Utah, at least as far back as 1925. The senior author has obtained information which indicates that psyllids have figured in potato culture in Washington County, Utah, at least since 1921.

ECONOMIC IMPORTANCE OF PSYLLID YELLOWS

Psyllid yellows in its effect upon the plant must be ranked among the most destructive of known potato diseases. If the plant is attacked when young, prior to tuber formation, no crop results, and early death of the plant frequently ensues. When the plant is attacked during early stages of tuber formation and prior to maturation, serious injury follows, and the resulting crop is of little value. If the plant is attacked after the tubers are well formed, the tubers may sprout, giving rise to new vines (fig. 1, A, B, C), or to sprouted, knobbed, or otherwise malformed tubers, of greatly reduced market value. (Fig. 1, B, C.)

Total crop failures from this disease in individual plantings are not uncommon. In 1927 experimental plots at the Davis County Experimental Farm yielded 40 pounds of marketable tubers from an area estimated to produce from 40 to 50 bushels under normal conditions. In Bountiful, Utah, 250 pounds of marketable tubers were obtained from a 1-acre field, and many of the most successful growers in this same district lost their entire crop, leaving their fields unharvested.

During this same year the disease was so severe that total crop failures resulted in entire valleys. Linford states that in Washington County the entire crop was destroyed. This county was surveyed late in August, long after potatoes are normally harvested, but there was no harvest in 1927, so complete was the destruction.

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[11] SHAPOVALOV, M. PSYLLID YELLOWS (CAUSE UNDETERMINED). U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 68: 29-30. 1929. [Mimeographed.].

[12] METZGER, C. H., and BINKLEY, A. M. Op. cit.

[13] LINFORD, M. B. Op. cit. See footnote 6, second reference.

Duchesne and Uintah Counties had approximately 50 per cent of a crop at the time the survey was made.

Davis and Weber County growers, who produce early potatoes chiefly, suffered the heaviest financial losses. Based on the acreage planted, it was estimated that 740 cars would have been shipped from Weber County; 110 cars were actually shipped, thus involving a loss of approximately 630 cars. Valued at $420 a car, this amounts to $264,600. Sixty-four cars of seed were shipped into the county at an average of 4.5 cents a pound, making a total outlay of $92,000,

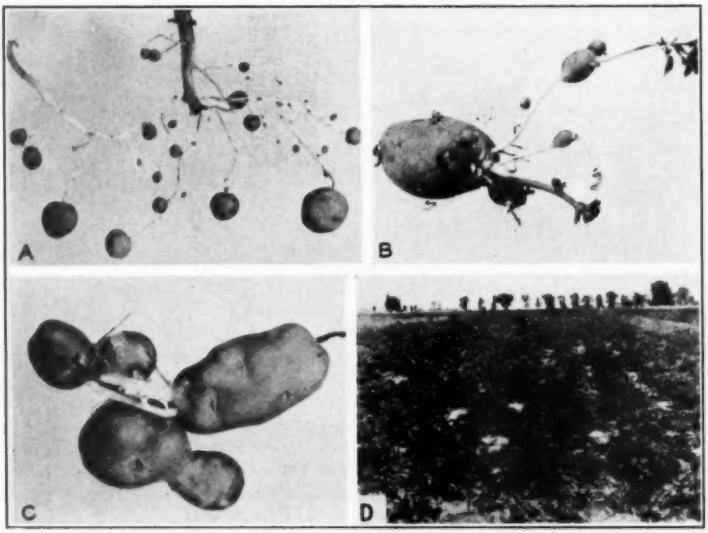

FIGURE 1.—A, Bliss Triumph affected with psyllids showing excess tuberization. One stolon has failed to terminate in tubers and has given rise to new shoots. Several shoots of this type may arise around a single infected mother plant. Note large number of secondary stolons terminating in tubers of various sizes. B, Sprouting of immature Russet Burbank tuber induced by psyllid feeding on parent plant. Practically all buds, both on the mother and the secondary tubers, have become active and have given rise either to new shoots or to a third crop of tubers. C, Sprouting of Russet Burbank tuber characteristic of this variety when parent plant is affected by psyllid nymph late in the season. Such sprouts may develop directly into tubers or may grow through the soil and give rise to second growth in the late-potato districts as in 1927. D, Five-acre field of large, vigorous, and apparently healthy Bliss Triumphs photographed October 4, 1927. These plants grew from small tubers left in the soil during the harvest of the early crop. The first crop was planted April 15 and yielded but 20 sacks per acre; on August 10 it showed 100 per cent psyllid yellows. With the exception of about 15, all plants of this 5-acre field were healthy and apparently free from psyllid yellows at the date the photograph was taken. Psyllid nymphs were present on the 15 plants showing symptons. Seed selected from this late crop gave progeny which were entirely free from any psyllid-yellows symptoms

or $46,000 more than the total value of potatoes actually shipped out. While exact figures are not available, observational data indicate that Davis County suffered even more acutely than Weber County owing to more complete destruction of the crop and to the higher early market prices of potatoes. These two counties lost fully 75 per cent of their crop.

The season of 1927 was especially favorable for potato production in Utah. An average annual acre yield of 185 bushels or more might have been expected, whereas only 135 bushels were realized, entailing a loss of from 25 to 30 per cent of the crop, or approximately

$750,000. The early crop in the State was practically a total failure. The late crop was less severely damaged although greatly reduced in market value. In certain areas little, if any, decrease in yield resulted, while in others isolated fields were seriously damaged. The malformation of the tubers of the late-maturing varieties, the Russet Burbank and the Rural, was of serious consequence. Linford estimated that from certain Russet Burbank fields in Cache Valley not more than 25 per cent of the tubers would pass as United States No. 1, and a local buyer reported that he was unable to find a single car of suitable Russet Burbank potatoes in the entire county.

Metzger and Binkley [14] state that in the Fruita district of Mesa County, Colo., which ships annually about 600 carloads, but 2 carloads were shipped as a result of the heavy infestation in 1927.

Losses from psyllid yellows in Utah during 1928 were greatly reduced as compared with those in 1927. Richards and his coworkers reported for 1928 that while the potato psyllid was almost coextensive with potato culture in the State, the damage was light as compared with the losses in the same areas in 1927, and that the total loss for 1928 would probably not exceed 7 per cent.

Damage to the potato crop during the year varied greatly in the different districts within the State. In Washington County, as in 1927, the destruction was again complete, and the entire crop planted between February 15 and March 10 was plowed under by June 10, except for two experimental plots. Davis and Weber Counties suffered losses between 10 and 12 per cent during the season. In a survey of the Hunter and Pleasant Green districts of Salt Lake County made during August, 1928, 72 per cent of the fields showed psyllid yellows. An average of 9.5 per cent of plants in all the fields visited showed the malady.

The disease occurred in other potato-growing areas, although there exists but little data to indicate the degree of loss. In Cache County the early garden crop was largely destroyed, and material damage was done in many of the late plantings. Sanpete and Sevier Counties were reported to have suffered severely.

Metzger and Binkley [14] report that psyllid yellows was not so serious in 1928 as in either of the previous seasons and that possibly 10 per cent of the acreage was infested so severely that it was plowed under and planted to other crops. The remaining acreage showed a degree of infestation varying from 0 to approximately 10 per cent.

Psyllid yellows was found in a number of areas in Utah in 1929; however, serious loss, so far as is known, was confined almost entirely to Washington County where upwards of 75 per cent of the crop was destroyed.

Psyllid yellows appeared generally throughout Utah in 1930. Reports of damage were obtained from every potato district in the State, although the damage in most areas was relatively light as compared with that of 1927. Washington County, as was the case for the last six years, again lost the major portion of its potato crop, and Utah, Davis, Weber, and Boxelder Counties sustained heavy losses to the early crop. It is significant that the early crop in these four counties, which comprises 85 per cent of the plantings, has been

[14] METZGER, C. H., and BINKLEY, A. M. Op. cit.

seriously reduced in yield for three of the seasons since 1927. Psyllid yellows was reported from the Twin Falls and the Idaho Falls districts in Idaho.

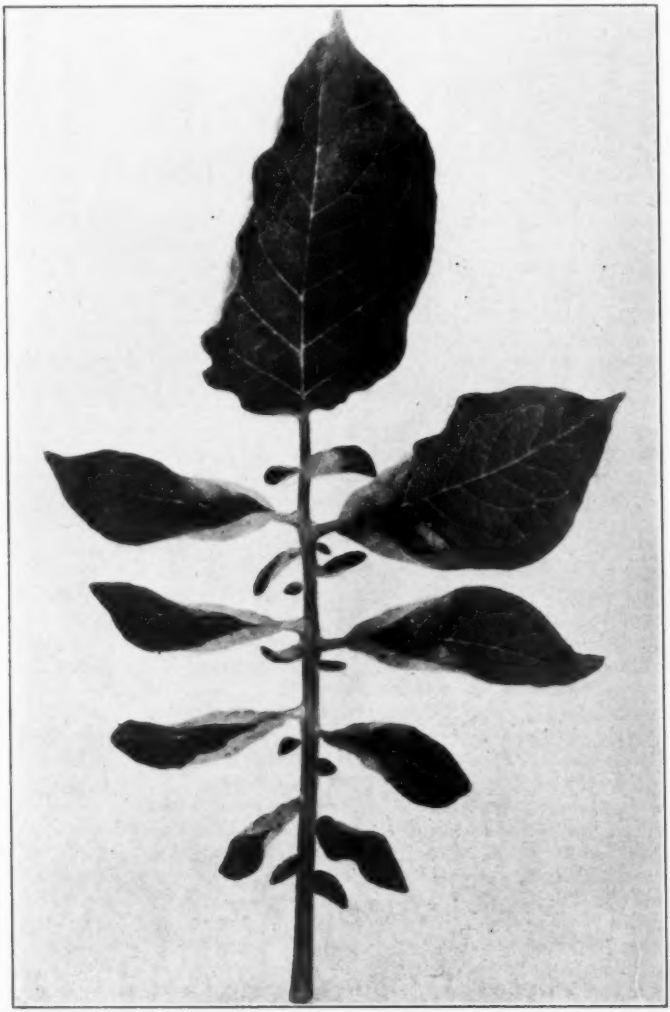

FIGURE 2.—Irish Cobbler potato leaf showing characteristic leaf symptoms of psyllid yellows. Notice the basal rolling and cupping of leaflets. These are accompanied by a characteristic yellowing and purpling, which is a typical expression of the disease on all varieties of potatoes. (Results of insect feeding under natural conditions in field)

During May and early June, 1931, psyllids appeared in great numbers in all early potato-growing areas in Utah, producing psyllid yellows in such quantities as to indicate possibilities of a general

epidemic. With the extreme heat and drought of middle and late June, however, the insects disappeared, and recovery from the disease was general. But little damage to either the early or late crop resulted. Psyllid yellows was reported severe, however, throughout the western slope of Colorado.

SYMPTOMS OF PSYLLID YELLOWS

SYMPTOMS UNDER CONDITIONS ACCOMPANIED BY SUMMER-LIGHT RELATIONS

Psyllid yellows is systemic and affects the form and physiology of the entire plant. A marginal yellowing and an upward rolling or cupping of the basal portion of the smaller leaflets on the younger leaves comprise the first symptoms of diagnostic value in the field.

FIGURE 3.—A, Psyllid yellows on Irish Cobbler potato showing characteristic symptoms of the disease induced by the feeding of 200 nymphs from eggs hatched in Petri dishes: nymphs had not fed on diseased plants before transfer to the plant. Note the typical leaf cupping and particularly the peculiar upright and rigid position of young leaflets, also apical hypertrophy and axillary growth. B, Apical hypertrophy of Irish Cobbler shoot induced by confining nymphs to older leaves by means of cloth bags. Note extreme cupping of leaflets and yellowing of cupped portions

(Figs. 2 and 3, A, B.) When diseased plants are exposed to intense sunlight, this basal cupping becomes pronounced. Leaflets affected in this manner tend to curve upward over the petiole and to assume an erect position quite distinct from the normal. (Fig. 3, A.) In the Bliss Triumph and the Irish Cobbler, and to a certain extent in all varieties, the rolled portions, and frequently other aerial portions of the plant, assume a distinct reddish or purplish color which may become so pronounced in cases of severe infection as to give a purplish tint to the entire field. In the more advanced stages of the disease the older primary leaves roll upward over the midrib, become yellow, develop necrotic areas, and degenerate rapidly (fig. 4, B), and as a result a plant is produced which consists principally of secondary leaves and branches supported by the primary stems.

Promptly on the inception of the disease, the aerial shoots usually suffer a sharp delimitation in stem elongation. The nodes enlarge, and the lateral buds are stimulated into activity and may develop

into axillary tubers (fig. 3, A, B, 5) or into stocky shoots capped with a rosette of small malformed leaves, which when fully developed give the plant a compact pyramidal shape, scarcely to be recognized as that of a potato plant. (Fig. 5, A, B.) With the enlargement of the nodes and subsequent growth of the axillary buds, the subtending leaves assume a position approximately at right angles to the stem instead of the acute angle characteristic of a normal plant. (Fig. 4, D.) The apical portion of the stems, including the terminal bud

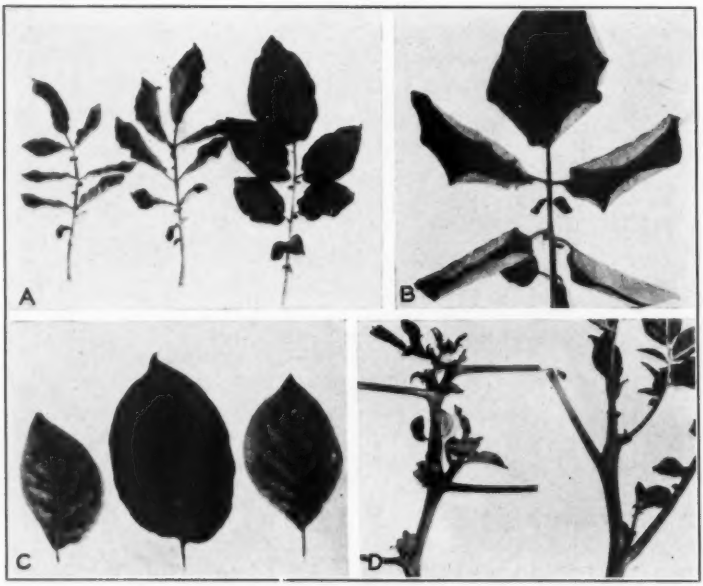

FIGURE 4.—A, Rolling and cupping of young leaflets of Russet Burbank potato very characteristic of the advanced stage of the psyllid yellows on this variety (left and center); normal leaf to the right. B, Effect of psyllid yellows on leaves which were mature at the time of the psyllid infestation. The leaves are somewhat papery and brittle in texture. Under field conditions necrosis sets in rapidly, and death soon results. This is characteristic of all varieties when affected by psyllids. C, Leaf chlorosis characteristic of psyllid yellows when affected plants are grown in the shade or during the short days of late autumn and winter. Affected leaves at left and right show distinct interveinal yellowing, also a distinct change in shape owing to an inhibition of the growth of the basal portion of leaflets, due apparently to the early destruction of chlorophyll in this region. Normal leaf in center. D, Irish Cobbler shoots showing modification of angle of leaf axis, also nodal enlargement characteristic of psyllid yellows. Left, diseased shoot (natural infection); right, healthy shoot

and frequently the first nodes and adjacent internodes, often become involved in a pronounced hypertrophy. (Fig. 3, A, B.)

If plants are attacked when young, stolon formation and tuberization are definitely suppressed, with the result that few, if any, tubers are set. When older plants are affected, the result is quite the reverse, and stolon development and tuberization are stimulated. The terminal buds of the stimulated stolons may fail to produce tubers and instead may grow directly through the soil, giving rise to a second crop of shoots adjacent to the mother plant. (Fig. 1, A.) The lateral buds along the primary stolon may develop directly into tubers (fig. 1, A), or they may give rise to secondary stolon branches which

FIGURE 5.—A, Characteristic pyramidal shape assumed by the Irish Cobbler potato in the more advanced stage of psyllid yellows. This peculiar form results from inhibited linear growth and concomitant extension of the lateral secondary shoots that arise from the axis of the old primary leaves. B, Advanced stage of psyllid yellows on the Irish Cobbler. A few primary leaves in advanced stage of deterioration are shown still attached to the primary stem. The apical portion of the plant is shown to consist essentially of secondary leaves and shoots grown from the axis of the primary leaves which have died. In the more advanced stage of the disease, the plants consist primarily of secondary leaves and shoots attached to the primary stem. C, Effect of psyllid yellows on the Early Ohio potato. Primary shoots elongate rapidly, resulting in small, badly curled leaves and axillary growth which is much distorted and usually of yellowish and reddish color. Note position and peculiar curling of the older leaves matured prior to psyllid infestation. This abnormal elongation is peculiar to the Early Ohio variety

in turn may provide opportunity for tuber formation, thereby resulting in development of numerous small potatoes. (Fig. 1, A, B, D.) Such tubers seldom reach a marketable size and may sprout directly without going into a rest period. (Fig. 1, B, C.) Such sprouting may give rise to new stolons, to leafy and apparently normal shoots, or to a variety of malformed tubers. (Fig. 1, B, C.) Discarded tubers from early harvest often produce a second crop of healthy vigorous vines. (Fig. 1, D.)

Plants affected after tubers have entered the early stages of dormancy may resume growth and produce stolons and many small tubers. The more mature tubers, if still attached to the parent plant when it is attacked, may also commence growth and develop into tubers of undesirable shape and low market value. (Fig. 1, C.) This breaking of the rest period in tubers after dormancy has been established and the inhibition of its establishment in developing tubers provides one of the constant and outstanding features of psyllid yellows.

SYMPTOMS UNDER CONDITIONS OF REDUCED LIGHT INTENSITY

The types, vigor, and sequence of symptomatological expression vary so greatly under both the intensity and the duration of light that symptoms characteristic of the open field are of little value in judging results under protected conditions in the greenhouse or under conditions of relatively dense shade. This fact was first suggested from observations made during the winter of 1927. Subsequent study, however, has provided a basic symptomatology for the study of psyllid yellows under shaded conditions and during the late fall, winter, and early spring months, and under cloth cages during the summer months. In fact, this symptomatology appears quite as reliable as that under natural conditions in the field and has been used as a criterion of judgment for all work conducted under cloth or under winter greenhouse conditions.

With decreased light intensity and duration, basal cupping and coloration do not occur uniformly. Instead, the basal lobes of the young leaflets turn distinctly yellow and are inhibited in growth, giving rise to linear leaflets quite distinct from those of the normal leaf. (Figs. 3, A; 4, C.) Yellowing finally appears in the interveinal portions of the affected leaflet, giving a general leaf chlorosis, seldom seen under field conditions. (Fig. 4, C.) A similar type of interveinal yellowing develops progressively from the younger to the older leaves, advancing slowly in later stages and showing a typical interveinal chlorosis in the most advanced stages of the trouble. The necrotic areas characteristic of the late stages of leaf deterioration in the open field have not as yet been observed under the more protected conditions. On the whole, the rate at which the plant succumbs to the disease under conditions of reduced light intensity and duration is much slower than that found in the open field; diseased plants under such conditions may survive quite as long as and sometimes longer than the healthy individuals.

The degree and type of bud activity both below and above the ground appear to be affected but little with varying light conditions.

NATURE AND CAUSE OF PSYLLID YELLOWS

RELATION OF THE PSYLLID TO THE DISEASE

During the early survey studies in June, 1927, Linford[15] first noted a small scalelike insect on plants showing symptoms of psyllid yellows. Subsequent studies showed a high correlation between the occurrence of the insect, which was later determined to be *Paratrioza cockerelli* by the Bureau of Entomology (fig. 6, A, B), and the characteristic symptoms of the disease. Survey studies throughout 19 counties in Utah showed this parallelism to be complete. Even in the most remote and isolated fields the disease and the insect were found constantly associated. In August, 1927, M. B. McKay, in a personal letter, reported the disease from Montana and southern Idaho, and in all cases the disease was associated with the insect. A

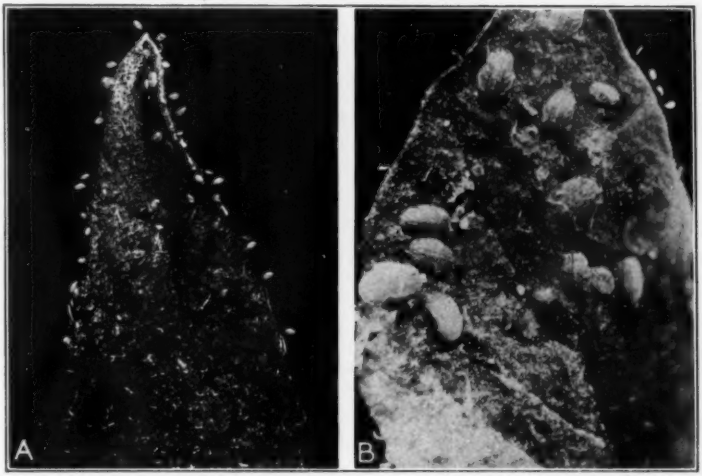

FIGURE 6.—A, Eggs of *Paratrioza cockerelli* Sulc on young potato leaflet. × 9. B, Nymphs of *P. cockerelli* feeding on young potato leaflet. Note eggs on leaf margin and scattered irregularly over the surface of the leaf. × 9

similar report was obtained from H. G. McMillan for Wyoming and Colorado. Such observations indicated rather clearly that the tomato psyllid was definitely concerned in the etiology of the disease.

On July 15, psyllids were taken from Davis County and placed on 50 plants in the pathological garden at Logan and covered with gauze insect cages. All plants developed the disease. Later, however, the disease spread throughout the ⅛-acre experimental plot, finally involving 100 per cent of the exposed plants. Forty plants in this plot, however, had been grown under insect cages and were kept covered until October 15, and 39 of the 40 remained entirely free from the disease. During the season a triangular rent was made in one of the cages, through which parts of the covered plant protruded. This plant became infested with insects and developed a clear case of psyllid yellows.

[15] LINFORD, M. B. Op. cit. (See footnote 6, first reference.)

Five experiments to test the etiological relation of *Paratrioza cockerelli* were made during September and October, 1927. On September 5, 40 psyllids were confined on each of three plants grown in the greenhouse under celluloid cages. Similarly, caged plants free from insects were held as controls. By September 9, one of the plants with insects had developed definite basal leaf rolling, a second plant had developed symptoms by September 18, and a third by October 2.

On September 9, 30 nymphs were transferred to each of 8 plants grown under celluloid cages. Three plants covered with cages but free from the insects were used as controls. By September 28, all plants on which the insects fed had developed advanced stages of the psyllid yellows disease. The controls remained normal.

On September 20, 30 nymphs were placed on each of 3 plants under celluloid cages; 2 plants were kept for controls. By September 30, 10 days later, 2 of the plants had exhibited marginal yellowing and basal rolling, and the third had developed the disease by October 10. The 2 control plants remained disease-free until discarded on November 1. Another series was started September 21 in which 30 insects were transferred to each of 7 plants. Four plants were held as controls. By October 10, the 7 insect-infested plants had developed clear-cut symptoms of the disease. The controls at this date were free from symptoms and insects. Later 1 of the check plants was found infested with nymphs and subsequently developed the disease.

In the fifth series plants were started in the greenhouse without cages. These plants were grown in 8 inches of soil on the greenhouse bench. All plants were in a vigorous growing condition on September 23, at which time the transfer of insects was made. Twenty plants distributed uniformly throughout the bed were employed and nymphs were carefully placed on 1, 2, or 3 leaves of each of the plants. The nymphs were confined to the leaves with gauze bags in such a way as to maintain complete isolation of other parts of the inoculated plant and of the control plants. Thirty insects were used to each plant; 51 plants were left as controls. Of the 20 to which insects were transferred, 3 showed marginal yellowing and basal leaf rolling within nine days. On October 10, 15 plants exhibited basal rolling as well as the characteristic yellowing and reddening. By November 1, when the experiment was terminated the plants had developed the disease and exhibited the various symptoms characterizing psyllid yellows in the field. The 51 controls remained disease-free. Nineteen diseased and nineteen healthy hills were selected and the tubers dug and weighed to determine the effect of the disease on tuberization. The data are summarized in Table 2.

TABLE 2.—*Effect of psyllid feeding on tuberization of Irish Cobbler potatoes in preliminary experiments*

Condition of plant	Hills	Tubers	Average tubers per hill	Average total weight per hill	Average weight per tuber
	Number	*Number*	*Number*	*Grams*	*Grams*
Diseased	19	317	16.6	75.5	4.5
Healthy	19	79	4.2	92.5	22.0

In these preliminary tests all phases of the disease as found in the field were produced under controlled experimental conditions, following an incubation period of six to nine days. The results justify the conclusion that the disease in some way is induced during the feeding process of nymphs of *Paratrioza cockerelli*.

RELATION OF NYMPHAL FEEDING TO THE DISEASE

Relation of the Number of Nymphs to Symptom Expression

In the preliminary tests typical symptoms of psyllid yellows were produced consistently with as few as 30 active nymphs. In late autumn what were considered characteristic symptoms of the disease failed to develop in the greenhouse on plants carrying as many as 60 to 75 nymphs. The following March, however, the progeny of these insects which had failed in the winter to induce psyllid yellows again consistently induced the disease. At first the cause for this apparent lack of potency on the part of the insect was not clear. In the course of the experiments, however, it became apparent that both the intensity and the duration of sunlight in some way either influenced the insect in its feeding or determined the type of symptom expression.

During 1928 and 1929 attempts were made to determine more accurately the quantitative relation between nymphal feeding and the disease. On August 11, three series of experiments were started in the field under gauze cages, using 10, 15, and 30 nymphs per plant. The time of feeding varied from 3 to 15 days, after which time the insects were killed by fumigation. The results are given in Table 3. All plants on which psyllids fed showed the early symptoms within 9 to 15 days after the insects commenced feeding. A study of the subsequent development of the disease on these plants was rendered valueless because of the general psyllid infestation of the field.

Table 3.—*Relation between number of psyllid nymphs and the length of feeding period and the first symptoms of psyllid yellows under cages in open field*

[Period of feeding, 3 to 15 days, commenced August 11, 1928]

Series No.	Plants	Nymphs per plant	Length of feeding period	Plants diseased	Series No.	Plants	Nymphs per plant	Length of feeding period	Plants diseased
	Number	*Number*	*Days*	*Number*		*Number*	*Number*	*Days*	*Number*
1	4	30	3	4	3	2	10	5	2
	4	30	3	4		2	10	7	2
	4	30	7	4		4	10	10	4
	4	30	10	4		2	10	15	2
	4	30	15	4					
2	4	15	5	4					
	4	15	10	4					

Between April 22 and July 25, 1929, four separate series of tests were completed, using from 1 to 9 nymphs per plant. In these tests nymphs failed to produce psyllid yellows, although comparable numbers during the past three years have occasionally been noted to induce the disease.

Additional data were obtained in four sets of experiments conducted under greenhouse conditions between March 19 and June 25, 1929. The numbers and the distribution of the nymphs, together with a summary of results, are given in Table 4.

TABLE 4.—*Summary of four series of experiments, showing relation between numbers and distribution of psyllid nymphs and the symptom expression of psyllid yellows on the Irish Cobbler potato*

[Feeding period, March 19 to April 12, 1929; data taken 41 days after insect transfer, 12 plants used in each experiment]

Distribution of nymphs	Psyllids per plant	Plants diseased	Plants remaining healthy	Distribution of nymphs	Psyllids per plant	Plants diseased	Plants remaining healthy
Nymphs placed on one leaf	300	11	1	Nymphs distributed equally on three leaves—Continued.	30	4	8
	150	11	1		15	0	12
	75	7	5		6	1	11
	30	3	9	Nymphs distributed equally on six leaves	300	12	0
	15	3	9		150	12	0
	6	0	12		75	10	2
Nymphs distributed equally on three leaves	300	12	0		30	7	5
	150	10	2		15	1	11
	75	9	3		6	0	12

Results of all the various experiments indicate that under greenhouse conditions and during the shorter days of late fall or early spring the number of nymphs feeding, and possibly the distribution of the insect on the plant, are vital factors in the production of psyllid-yellows symptoms. Much additional work under controlled environmental conditions must be done to determine more accurately this quantitative relation of nymphs to the occurrence of the disease.

RELATION OF NYMPHAL FEEDING PERIOD TO THE PRODUCTION OF EARLY SYMPTOMS OF THE DISEASE

Experiments were started on March 6, 1930, to determine the shortest period of insect feeding necessary to produce psyllid yellows. In the experiment, 200 nymphs (100 on each of two leaves) were confined on each of 20 healthy plants. Insects were removed at intervals as follows: Plants 1–5, after 2 days; plants 6–10, after 3 days; plants 11–15, after 4 days. Insects on plants 16–20 were allowed to feed for the entire period of 36 days. Ten plants, Nos. 21–30, were kept free from insects and remained free from the disease throughout the entire experiment. This experiment was repeated with essentially the same results. The results are recorded in Table 5.

Results indicate that at least three days' feeding is necessary to produce the first definite symptoms of psyllid yellows. Four days' feeding gave a more vigorous expression of the early symptoms and resulted in a more permanent effect, although with a 4-day period the disease did not progress far beyond the early manifestation.

This failure of symptoms to appear as a result of fewer than three days' feeding was not occasioned by any peculiar masking effect of the environment, as the early appearance of the disease resulted when the same number of insects fed for longer periods, and a complete expression of symptoms was produced by nymphs feeding continuously for a period of 36 days.

TABLE 5.—*Relation between short periods of nymph feeding and the production of early or first symptoms of psyllid yellows in the Irish cobbler potato*

[Feeding time 2, 3, 4, and 36 days; 200 insects used on each plant]

Plant No.	Feeding period	Condition of plant at removal of nymphs	Condition of plant 10 days after feeding commenced	Condition of plant 36 days after the beginning of nymph feeding
	Days			
1	2	No symptoms.	Normal	Large plant apparently normal.
2	2	do	do	Very slight marginal yellowing, otherwise normal.
3	2	do	do	Do.
4	2	do	Mere trace of marginal yellowing.	Slight basal yellowing and restriction, otherwise normal.
5	2	do	Normal	Slight basal yellowing, otherwise normal.
6	3	do	Slight interveinal yellowing.	Basal, marginal, and interveinal yellowing distinct early symptoms.
7	3	do	Normal	Normal except slight yellowing of young leaves.
8	3	do	Slight trace of yellowing on young leaflets.	Slight yellowing of 3 leaves, otherwise normal.
9	3	do	Normal	Young leaflets yellow and some interveinal yellowing could be considered type of early symptoms.
10	3	do	Slight marginal yellowing.	Distinct basal yellowing on a number of younger leaves.
11	4	do	Normal	Do.
12, 13, 14	4	Symptoms	Early symptoms of disease; basal, marginal, and interveinal yellowing.	Distinct basal, marginal, and interveinal yellowing on younger and older leaves, showing that feeding had some effect on subsequent growth.
15	4	do	Marginal, basal, and interveinal yellowing.	
16, 17, 18, 19, 20	36	do	Marginal, basal, and interveinal yellowing; all symptoms exhibited.	Full expression of symptoms of disease above and below ground.
20–30	0	Healthy	Healthy	Healthy, with occasional plants showing slight yellowing of younger leaflets.

RELATION OF NYMPHAL FEEDING PERIOD TO THE SEQUENCE AND DEGREE OF SYMPTOM EXPRESSION

During the earlier greenhouse and field studies instances were observed frequently which indicated the existence of a definite relation between the length of the feeding period and the final degree of disease expression. In many cases, especially under greenhouse conditions, apparent recovery was noted, and in general the accumulated evidence suggested that continued feeding of nymphs was necessary to produce the full expression of psyllid yellows.

On February 23, 1930, two series of experiments were started to determine more accurately the relation of the feeding period to the early appearance and to the continued development of symptoms of psyllid yellows. Twenty plants, exclusive of controls, were used in each of the two series. In series 1, 200 nymphs (100 on each of two leaves) were confined by means of gauze bags to each of 20 healthy plants. The insects were then removed from the plant by clipping off the infested leaf as follows: Plants 6–10, after 12 days' feeding; plants 11–15, after 16 days, and plants 16–20, after 26 days' feeding. Plants 1–5, inclusive, were fed upon for 48 days.

Series 2 was set up in the same manner as series 1. Plants 1–5, inclusive, were fed upon for a period of 50 days, or during the entire time the experiments were run. Insects were removed as follows:

Plants 6–15, after 9 days; plants 16–20, after 14 days. Eight insect-free plants were used as controls for series 1 and nine plants for series 2. All plants in both series were grown openly in the greenhouse bench in 8 inches of soil. A summarized statement of series 1 and 2 is recorded in Table 6. (Fig. 7, A, 1–5.)

TABLE 6.—*Summary of results of experimental series 1 and 2 in which 200 psyllid nymphs were fed on Irish Cobbler potato plants for various periods longer than the incubation period*

[See text for details and also Figure 6A, 1–5

SERIES 1

Length of feeding	Plants involved	Average height	Average number of leaves per plant	Average length of leaves	Average tubers per hill	Average weight of tubers per hill	Average weight per tuber	General expression of symptoms at the end of the experiments, which lasted 50 days
Days	*Number*	*Inches*	*Number*	*Inches*	*Number*	*Ounces*	*Grams*	
0	5	16.0	10.3	10.3	4.5	6.7	42.2	Normal.
12	5	15.2	11.6	11	8.8	5.8	18.7	Some apparently normal; others slightly yellowed; some axillary growth.
16	5	14.2	13	9.9	8.8	4.05	13.0	Distinct interveinal yellowing; abundant axillary growth.
26	5	13.1	12	9.9	11.4	4.02	10.4	Clearly diseased; yellowing of younger and older leaves.
50	5	11.3	12	8.7	14.0	1.6	3.2	Severe disease of all plants; yellowing, reddening, and cupping of apical leaves; apical and axillary hypertrophy.

SERIES 2

0	8	14.3	.....	9.8	4.8	5.6	23.1	Normal.
9	10	13.7	.....	9.08	9.0	5.4	17.0	Some apparently normal; others slightly yellowed; some axillary growth.
14	5	13.3	.....	8.3	8.4	4.6	15.5	Distinct interveinal yellowing; abundant axillary growth.
50	5	12.8	.....	9.5	13.4	2.3	4.8	Severe disease of all plants; yellowing, reddening, and cupping of apical leaves; apical and axillary hypertrophy.

Most of the infested plants in the two series showed slight marginal and interveinal yellowing by the sixth day. All plants showed unmistakable symptoms at the end of the tenth day. Many plants exhibited marked rolling and cupping of the smaller and younger leaflets. Basal, marginal, and interveinal yellowing was pronounced on all plants from which insects were removed on the twelfth day in series 1 and by the ninth day in series 2. A number of these plants also showed basal leaf cupping and reddening of younger leaves. More advanced symptoms, such as leaf rolling, pronounced interveinal yellowing, slight nodal enlargements, and beginning axillary growth, were evident at the end of the sixteenth and twenty-sixth days.

In all cases, on the removal of the insects, the progress of development of the symptoms appeared to cease rather abruptly. All red and purple coloring that had developed during the feeding period on affected leaves completely disappeared. Leaf rolling or cupping ceased, and the rolled leaves uniformly unrolled and assumed normal

position and shape. A definite tendency toward recovery was noted also in both marginal and interveinal yellowing. Where but slight yellowing occurred prior to insect removal, recovery was apparently complete; however, when the yellowing was well advanced, the

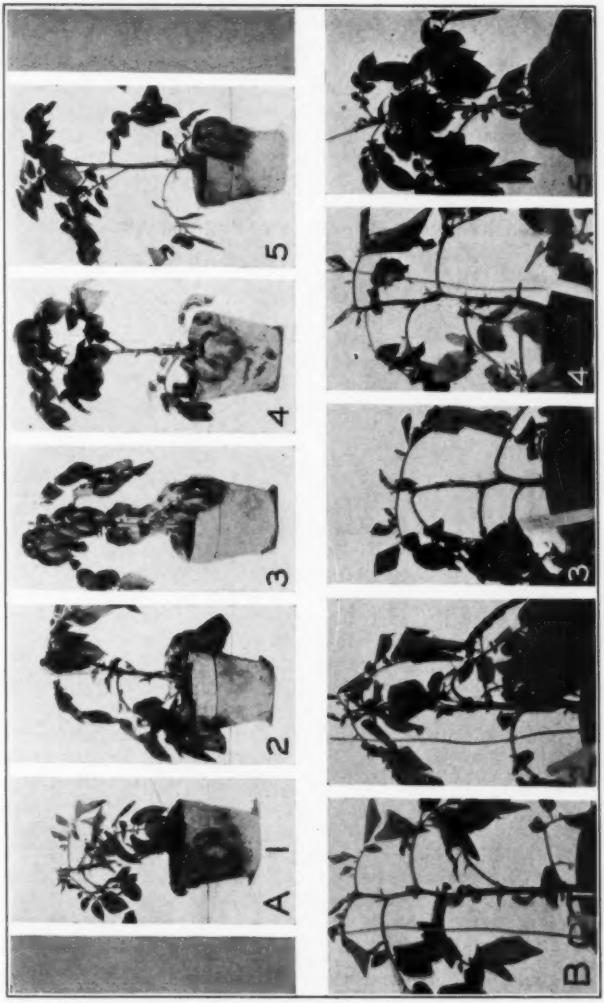

FIGURE 7.—A, Irish Cobbler potatoes on which psyllid nymphs have fed for varying periods. For data see page 200 and Tables 10 and 11. The feeding periods were as follows: Plant No. 1, 50 days; plant No. 2, 26 days; plant No. 3, 16 days; plant No. 4, 12 days; plant No. 5, no feeding (control). B, Psyllid yellows induced in Irish Cobbler plants by nymphs from eggs hatched in Petri dishes on normal potato leaves. Plants Nos. 1, 2, and 3 are representative of separate series on which nymphs from different broods, kept free from all diseased plants, fed. Plant No. 4, injured by nymphs from diseased potato plants. Plant No. 5, control, kept free from all sources of infection

normal green color was seldom regained. Plants fed upon for fewer than 16 days assumed practically normal linear growth and in many cases equaled the unaffected plants. Subsequently, axillary and apical growth was of a normal green color and of apparently normal texture. Certain physiological processes, however, were so disturbed

during the feeding that the plants did not completely recover from the effects of the disease. This is clearly shown in the relative height of plants (fig. 7, A), number of tubers per hill, average weight of tubers per hill, and average weight per tuber. (Table 6.) In all cases, a greater number of tubers with a decreased total hill yield and decrease in tuber weight resulted with increased length of feeding. All symptoms characteristic of the disease developed prominently in plants on which the insects were fed for the total 50 days. (Fig. 7 A, 1.)

Results indicate clearly that the symptom complex characteristic of psyllid yellows is entirely contingent upon the feeding process of nymphs of *Paratrioza cockerelli* and further that a complete expression of symptoms in their characteristic sequence and intensity, under greenhouse conditions, is entirely dependent on the continued feeding of the nymphs.

RELATION OF ADULT FEEDING TO THE DISEASE

Studies with nymphs have given no clue as to the exact effect of adult feeding on symptom expression. Two sets of experiments were set up July 16, 1929, in which adult psyllids were allowed to feed for a period of 28 days on healthy Irish Cobbler plants in the open field. In the first series, 50 adults were fed on each of 10 plants. These were confined in groups of 10 on each of 5 different leaves of the plant, by means of small spring cages. In the second series, 75 insects were fed on each of 10 plants, 15 in each spring cage on each of the 5 separate leaves. Five of the insect-infested plants of each series were then placed under insect-proof cages and kept covered for the entire time of the experiment. The other five plants of each series were left uncovered and exposed to the natural weather conditions in the field. Cages with insects were shifted to new feeding areas on the leaf every third day and the eggs laid during the period destroyed. All insects lost through death or escape were promptly replaced by additional insects from the insectary. No indication of the disease developed from adult feeding either during or subsequent to the 28-day period.

Five additional series of experiments were started on August 22 in the greenhouse to determine whether adults in numbers greater than those used in the earlier field studies could induce yellows. In these experiments the insects were confined to the plants in large celluloid cages under which the plants had been grown, thus allowing feeding over the entire plant surface. To insure against nymph feeding, the adults were removed at intervals of four days and all eggs destroyed. In this process, adults were removed from the cages by suction guns and placed in small vials. The eggs were then crushed and the original number of insects replaced. All adults used in the experiment were obtained from diseased plants in the insectary.

In series 1, 100 adults were placed on each of 10 plants on August 22 and allowed to feed until September 16, a total of 25 days. At the end of the feeding period, none of the plants showed symptoms of psyllid yellows. Eggs laid during the last 4-day period of insect feeding, September 12 to 16, were allowed to hatch on 6 of the 10 plants used in the experiment. All 6 of these plants showed typical symptoms within 9 to 12 days after the hatching of the eggs. The other 4 plants, kept free from nymphs, did not develop psyllid yellows after the removal of the adults.

Series 2 was a duplication of series 1 except that 150 instead of 100 adults were used. Eight of the ten plants remained free from symptoms for 28 days, at which time the experiment was terminated. Two plants showed unmistakable symptoms of disease. When examined, however, both diseased plants were found to carry a number of psyllid nymphs, hatched from eggs which were overlooked in the experiment. All adults were removed from the 10 plants at the end of 28 days. As in series 1, the eggs laid during the last 4 days of the experiment were allowed to hatch on 6 plants, all of which developed psyllid yellows within 8 to 12 days after the nymphs commenced feeding.

Series 3, 4, and 5 differed from 1 and 2 only in the number of adults used. In series 3, 200 adults were allowed to feed on each of 5 plants; in series 4 and 5, 500 and 1,000 adult insects, respectively, were placed on each of 3 plants. After 28 days' feeding, none of the 11 plants in the three series showed the slightest symptom of disease. Plants fed upon by these larger numbers, however, were evidently stunted in growth, and when the numerous nymphs from eggs laid during the last four days of the experiment were allowed to feed, 7 of the plants were killed.

The five experiments on adult feeding were carefully checked, (1) by placing 50 nymphs on 10 healthy plants and allowing them to feed during the 28 days, all of them developing psyllid yellows, and (2) by placing 7 plants under cages not exposed to insect feeding but treated as if eggs were being crushed at the same period as eggs were crushed in the experiments. Except for apparent stunting and occasional leaf mutilation, these latter plants showed no indication of treatment. A final check was provided by growing 10 plants under cages free from insects and untouched. The check plants remained free from disease during the 28 days of the experiment and grew somewhat larger than did those exposed to insects.

Results of the five series on adult feeding are given in Table 7.

TABLE 7.—*Results of five series of experiments in which various numbers of adult psyllids were fed for 25 days on healthy Irish Cobbler potato plants*

Experiment No.	Insects used per plant	Stage of insect	Plants used	Diseased plants	Healthy plants
1	100	Adult	10	0	10
2	150	do	10	[a] 2	8
3	200	do	5	0	5
4	500	do	3	0	3
5	1,000	do	3	0	3
Control	0	Nymph	20	20	0

[a] As explained in the text, these 2 plants showing unmistakable symptoms were found to have a number of nymphs feeding on them.

RELATION OF NYMPHS TO INFECTIVE PRINCIPLE

The failure of adult psyllids to induce the disease at once raises the question as to the origin or source of the substance injected by nymphs which produces the pathological symptoms characteristic of psyllid yellows in the potato. An attempt was made to separate the nymphs from this substance by removing eggs from the leaves of potato

plants to healthy disease-free leaves in Petri dishes where they were allowed to hatch. The resulting nymphs were then transferred to healthy plants on which they were allowed to develop into adults and finally to lay eggs. From these eggs three separate broods of nymphs (A, B, and C) were obtained and were placed on healthy potato plants in the greenhouse. Two hundred nymphs were placed on each plant used in the experiment. Two plants were used for insects from brood A, 3 for brood B, 4 for brood C. Nymphs were allowed to feed for 27 days. Eight plants were held free from insects as controls, and additional plants were inoculated, each with 200 nymphs from diseased potato plants. All nine plants fed on by the nymphs from broods A, B, and C developed uniformly symptoms entirely characteristic of psyllid yellows and in a more severe form than that produced by nymphs from diseased potato plants. The results are recorded in Table 8.

TABLE 8.—*Results from the feeding of psyllid nymphs which were obtained from eggs hatched in Petri dishes on disease-free potato leaves*

[Feeding period, 27 days, March 25 to April 22, 1929]

Source of insects	Plants employed	Insects per plant	Diseased plants	Healthy plants	Incubation period	Degree of severity of disease
	Number	*Number*	*Number*	*Number*	*Days*	
Brood A	2	200	2	0	6	Severe expression.
Brood B	3	200	3	0	6	Do.
Brood C	4	200	4	0	6–8	Do.
From diseased plants	8	200	8	0	6–8	Mild to severe; only 2 plants equalled in severity the plants fed upon by insects, broods A, B, and C.
Insect-free (control)	8	0	0	8	.....	

TRANSMISSION STUDIES

During 1928 and 1929 various standard methods used for the artificial transfer of virus diseases were employed in attempts to transmit psyllid yellows from diseased to healthy potato and tomato plants. Large numbers of plants in different stages of development were used as sources of inoculum and for inoculation. All these attempts gave negative results. Binkley (*1, v. 26*) also reports negative results in his attempts to transmit artificially the disease in the tomato. In preliminary experiments grafting has also proved ineffective as a means of transmission. To date, the nymph of *Paratrioza cockerelli* appears to be the only known means by which psyllid yellows can be transmitted from diseased to healthy plants, if such transfer actually takes place, or by which the disease is induced directly into healthy plants. Further work is now in progress to test all known methods of artificial transmission.

Observational and experimental evidence further indicates that psyllid yellows is not transmitted through the tuber from diseased parents to the progeny under field or greenhouse conditions in Utah. Young plants from attached tubers produced by diseased plants or from tubers detached from the mother plants show no symptoms of the disease; however, both may develop the disease with equal readi-

ness when fed upon by psyllid nymphs. It is also quite possible that young attached shoots may in time develop the disease through the mother plant, when the latter is fed upon continually by nymphs, although this relation has not been clearly established. In the field shown in Figure 1, D, only 18 plants were found to exhibit distinct psyllid symptoms on October 4, 1927, when the photograph was taken. Nymphs were found actively feeding on all of these. Three bushels of tubers selected from this same crop and planted in 1928 produced vigorous plants entirely free from any signs of the disease.

Because of the vigor of the plants, the owner selected seed from this same stock for his 1929 planting. Although in 1928 the plants were infested with the psyllids later in the season, the plants in 1929 again appeared entirely free from the disease and remained so throughout the season. Other observations similar to the foregoing were made during 1927 to 1930, inclusive, and plantings made for the purpose of determining this relation gave similar results. In 1929, 15 fields of second-crop potatoes from discarded infested tubers were observed to show no symptoms of psyllid yellows until fed upon by psyllid nymphs later in the season. Under no observed conditions have plants, grown from tubers produced by infected parents and kept free from insects, shown symptoms of psyllid yellows either in the greenhouse or in the field.

EPIDEMIOLOGY OF PSYLLID YELLOWS

GENERAL NATURE

The sudden appearance of psyllid yellows in 1927 provides an interesting problem for analysis. According to data already presented, the disease appeared rather suddenly in the early crop throughout Utah, the western slope of Colorado, southern Idaho, and local areas in Montana and Wyoming. This apparently sudden and extensive distribution was no less startling than the degree of uniformity with which the disease occurred. In Utah few, if any, fields in the infected areas escaped the disease, and in certain localities, particularly those growing early potatoes, infection was so complete that scarcely an unaffected plant could be found. Isolated fields in canyons many miles removed from the main valley centers of potato culture became infected before the end of the season. A similar situation existed throughout the western slope of Colorado where the disease almost completely destroyed the potato crop in many districts.

FACTORS INVOLVED

The sudden appearance and widespread and uniform distribution of psyllid yellows in 1927 are explainable only by the presence of some prolific and rapidly disseminated etiological factor, such as is found in the tomato psyllid. The distribution of *Paratrioza cockerelli* prior to 1927, the nature of the insect, that is, its natural means of dissemination, rate of development, longevity and degree of fecundity, insect parasitology, and the number and distribution of host plants are, therefore, vital factors. The various environmental factors, such as temperature, humidity, and air movement enter into the problem chiefly as a result of their influence on the fecundity and dissemination of the insect.

Distribution of the Insect Prior to 1927

The suddenness with which psyllid yellows occurred and the novelty of the disease in the infested areas during 1927 suggests an early migration of *Paratrioza cockerelli* from a few local areas in which the insect normally survived into the various parts of Utah, Idaho, Montana, Wyoming, and parts of Colorado. On the other hand, it is quite possible that the insect had previously occupied these infected areas but had occurred in such small numbers as to entirely escape notice, especially as an etiological factor; thus, with a disturbance of some peculiar balance, the involvement of the entire region of the 1927 epidemic was made possible.

Survey studies subsequent to 1927 have established the fact that *Paratrioza cockerelli* had been present and responsible for psyllid yellows during 1925 and 1926 in the Green River and Washington County districts in Utah and in certain districts in Colorado. It is conceivable that the insect might have spread from these various areas throughout the range occupied in 1927. The more general distributions of the insect reported by Crawford (*4*) in 1914 appears to furnish the more plausible explanation for the 1927 epidemic of the disease. This writer gives the distribution of the species of *Paratrioza cockerelli* as covering the entire southwestern part of the United States and reports the specific localities in which the insect has been found as follows: Boulder, Rocky Ford, and Canon City, Colo.; Milford and Park City, Utah; Tucson and Fort Yuma, Ariz.; Madagascar Mountains, N. Mex.; Claremont, El Centro, San Luis Obispo, Argus Mountains, Alameda, and Death Valley, Calif. The occurrence in Milford and Park City, Utah, is especially interesting, and the further fact that the Utah station entomologists have observed *Paratrioza cockerelli* in the infested areas in previous years suggests the distinct possibility that the insect might have been more generally distributed than had been supposed. The ability of the insect to overwinter in those areas to which it spread in 1927 and its early appearance in Davis County, Utah, apparently bear out this possibility.

Nature of the Insect

Crawford (*3*, *4*), Ferris (*7*), and Lehman (*10*) describe *Paratrioza cockerelli* as a small homopterous insect of the family Cheridae with a small, active clear-winged adult stage, varying in size from 1.3 to 1.8 mm in length and from 0.39 to 1 mm in width. This small adult is extremely active; when disturbed, it springs quickly into the air by means of its powerful hind legs. The springing habit of the insect, assisted by flight and by the wind, undoubtedly functions effectively in the distribution of the species and consequently in the spread of the disease at least within relatively localized areas. The insect is so minute that when once in the air it might readily be transported considerable distances by the air currents. It is conceivable that the combination of these factors may result in the transportation of the insect over long distances.

Observations as to the longevity of the adult vary. Compere (*2*) noted that *Paratrioza cockerelli* adults lived for three months in captivity. Knowlton and Janes (*9*) gave 64 days as the longest life period for an adult male and 189 days for an adult female, with an average life of 25.22 days for males and 34.4 days for females. The

writers found the life of the female to vary from a few hours to 60 days, with an average longevity under greenhouse conditions of 45 days. The long life of the insect may result in overlapping of broods, which fact, coupled with a long oviposition period, aids greatly in increasing the number of affected individuals.

Compere (*2*) observed that egg laying commenced three days after copulation, although he did not state the length of the preoviposition period. Knowlton and Janes (*9*) reported a preoviposition period varying from 5 to 25 days, with an average of 10.1 days from emergence of adult female until the first eggs were produced. Similar results to those of Knowlton were obtained by the writers. It will be noted from Table 9 that the preoviposition period for the 10 females varied from 4 to 20 days, with an average of 9.7 days between emergence and the production of the first eggs. Compere (*2*) stated that three adults in captivity laid eggs for a period of 3 days. Knowlton and Janes (*9*), on the other hand, gave a much longer period of egg laying, varying from a few days to a maximum of 179 days. They found the average oviposition period of 58 females to be 21.45 days. For 10 insects observed under greenhouse conditions, the writers found the oviposition period to vary from 20 to 53 days, with an average of 35.2 days. (Table 9.)

TABLE 9.—*Egg-laying history of 10 adult females of Paratrioza cockerelli (Sulc) under greenhouse conditions*

[One pair of adults was confined to leaves of potatoes by means of spring cages; at the end of each 24 hours the cages with insects were removed to a new leaf area and eggs laid during the 24 hours counted]

Adult No.	Pre-egg-laying period of adult	Length of egg-laying period	Total eggs laid	Adult No.	Pre-egg-laying period of adult	Length of egg-laying period	Total eggs laid
	Days	*Days*	*Number*		*Days*	*Days*	*Number*
1	5	20	430	8	20	35	553
2	6	21	458	9	17	31	497
3	11	33	851	10	5	36	641
4	16	28	504				
5	4	53	1,460	Minimum	4	20	430
6	6	46	979	Maximum	20	53	1,460
7	5	49	829	Average	9.7	35.2	720

In the shortest oviposition period of 20 days, as shown in Table 9, the insect laid 430 eggs, while the female with an oviposition period of 53 days deposited 1,460 eggs. Also, as shown in Table 9, the 10 insects averaged 720 eggs. Knowlton and Janes (*9*) report a total of 19,833 eggs from 60 females, with an average of 330.55 eggs for each of the 60 females. These writers also record a maximum of 1,352 eggs for a single female, laid during a period of 179 days.

Knowlton and Janes (*9, p. 285*) record a high percentage hatchability of psyllid eggs. Of 9,615 eggs observed, 7,989, or approximately 83 [16] per cent, hatched, giving rise to living nymphs. The large number of eggs, their hatchability, and the extended period during which the female psyllid may lay eggs undoubtedly plays an important part in the epidemiology of psyllid yellows.

Paratrioza cockerelli passes through an incomplete metamorphosis with the usual stages of adult, egg, and nymph. The time required

[16] This should be 83 per cent.

for completion of the life cycle has been variously estimated and has been shown to depend to quite an extent upon the influence of environment on the development of the insect during the various stages of growth. Compere (*2*) reports that approximately 45 days were evidently required to complete the life cycle under the conditions imposed in his experiments. He further states that the broods "are continuous throughout the year." Lehman (*10*) gives 25 days as the time necessary for the life cycle of the insect. Knowlton and Janes (*9, p. 286*), in laboratory studies with a much larger number of insects than observed by Compere, give the time for development of the various instars shown in Table 10.

TABLE 10.—*Time of development of various instars of Paratrioza cockerelli as given by Knowlton and Janes*

Instar	Nymphs observed	Average days required	Instar	Nymphs observed	Average days required
First	252	2.76	Fourth	151	2.72
Second	186	2.44	Fifth	133	4.87
Third	158	2.49			

Knowlton and Janes (*9*) state that the total time for nymphal development for some 800 nymphs observed varied with conditions from 12 to 21 days, with an average of 16 days. These data correspond essentially with the observations of the present writers. Judging from the data supplied by Knowlton, the life cycle of *Paratrioza cockerelli* from egg laying to adult emergence may be completed within from 25 to 37 days, depending on the conditions to which the insect is exposed. These data are significant in view of the fact that nymphs have been observed under the rather vigorous climate at Logan, Utah, as early as May 4, and in an active state as late as November 2. In view of these data, it appears possible that from three to five broods may develop during a single season. Consequently, the progeny from a single female adult under favorable conditions may number into the millions in a single season. In view of such fecundity and length of life favorable ecological conditions for overwintering and propagation may initiate an epidemic any season.

PARASITOLOGY OF INSECT

Little is known of the parasites of *Paratrioza cockerelli*, although the writers have observed that such do occur and undoubtedly play an important part in the survival of the species. Whether the absence of psyllid parasites played a significant part in the epidemic of 1927 and in the persistence of the insect during 1928–29 and 1930–31, there is no way of determining. However, as the variation in the physical environment appears inadequate to explain the epidemic of 1927, it would seem that the abundance and sudden appearance of the insect during the particular year might be adequately explained by a serious disturbance of the biological balance which ordinarily keeps the insect in check. The parasitology of *Paratrioza cockerelli* remains as a problem for further study.

Number and Distribution of Host Plants

The food plants of *Paratrioza cockerelli* are undoubtedly important in facilitating the distribution of the insect. The degree of influence which these food plants exert, however, is still problematic, although the number of plants on which the insects have been found is significant. Crawford (*4, p. 72*) lists food plants as follows: "Pepper (*Capsicum annum*), tomato (*Solanum nigrum*), potato (*Solanum tuberosum*), * * * spruce (*Picea* sp.) pine (*Pinus monophylla*), alfalfa (*Medicago sativa*). List (*11, 12*) reports psyllids as being abundant on the cultivated tomato in Colorado.

Ferris (*7*) observed adults and nymphs from California on tobacco (*Nicotiana tabacum*), and Compere (*2*) stated that the insects on which he made observation in the Golden Gate district of California were found on the Jerusalem cherry (*Solanum capsicastrum*). Van Duzee (*17*) listed the insects on members of the following genera: Capsicum, Solanum, Purshia, Picea, Pinus, and Medicago. Essig (*5, p. 438*) in 1917 added *Datura* sp. and *Solanum nigrum* to the list of host plants. The writers have found *Paratrioza cockerelli* nymphs and adults feeding abundantly on tomato (*Lycopersicum esculentum*), groundcherry (*Physalis longifolia*), matrimony-vine (*Lycium vulgare*), and on all varieties of the potato (*Solanum tuberosum*) grown in Utah during the past three years. In the greenhouse the nymphs have been found to feed on tobacco.

The common groundcherry appears to be a most favorable host of *Paratrioza cockerelli* in Utah, second possibly only to the potato in importance. In 1927, this plant was universally infested with psyllids throughout the State, and even in isolated areas miles from potato fields it was found to support large numbers of both nymphs and adult psyllids. The general distribution of this Physalis species in Utah undoubtedly facilitated the dissemination of *P. cockerelli* in 1927 and figured as an important factor in the epidemic of psyllid yellows in that year, as well as in the general epidemiology of the disease.

DISCUSSION

Accumulated evidence clearly indicates that *Paratrioza cockerelli* in some definite way is involved in the etiological complex of psyllid yellows. Exactly in what manner the insect produces the disease, however, has not been revealed, although some facts are sufficiently suggestive as to merit definite consideration. The total absence of visible disturbances at the point of insect feeding, together with the systemic nature of psyllid yellows and the few insects necessary to induce the disease, precludes mechanical injury or food extraction as possible elements in disease production. On the other hand, these basic relations considered in connection with other features of the disease suggest more definitely that the disease is produced either by a virus, transferred from plant to plant, or by some toxic substance injected into the plant tissue during the feeding process of *P. cockerelli* nymphs. Facts have been obtained which support both possibilities.

The more critical studies have revealed facts which are difficult to fit into the virus theory. The abrupt cessation of symptom development with the removal of the insect followed by an apparent

uniform tendency to recover from the disease, is especially pertinent in this connection. These facts, when taken together with the development of apparently healthy organs from axillary buds and the absence of tuber transmission of the disease, renders acceptance of the virus concept particularly difficult. In this connection it is also necessary to recall that the adult psyllid is incapable of producing psyllid yellows after feeding on diseased plants, and that the infective principle, whatever its nature, is probably inseparable from the psyllid nymphs, which alone are capable of producing the disease. The foregoing, however, must be considered in view of the fact that Shapovalov (*16*) reports tuber transmission of psyllid yellows and that Binkley (*1*) claims to have separated that which he considers as a virus from the nymph and has unhesitatingly designated the disease as of virus origin. On these two points the meager data presented by these workers raise a serious question as to the justification for their conclusion. Whether or not the substance injected into the potato plant by *Paratrioza cockerelli* is a virus remains a question for future research.

To the writers the explanations that appear most plausible, especially in view of a number of the foregoing facts, is that *Paratrioza cockerelli* during its feeding processes injects into the plant tissues some toxic substance which quickly becomes systemic and possibly produces the exaggerated responses characteristic of the disease by its interference in some way with the carbohydrate metabolism of the plant.

It is interesting in this connection to note that Monteith and Hallowell (*13*) report a condition involving the feeding of leaf hoppers on legumes which resemble closely the etiological complex and symptomatological responses involved in psyllid yellows. They suggest that the pathological symptoms involved are probably the result of some "chemical or enzymatic toxin," secreted by the leaf hoppers.

Should the toxin theory of psyllid yellows prove correct, it would appear that this disease, also the whitetop of alfalfa and other legumes, the hopperburn of potatoes, and possibly other diseases, constitute a group of plant maladies that may truly be designated, based on their peculiar etiology, as insect diseases.

SUMMARY

Psyllid yellows as a disease of the potato first came to the attention of plant pathologists in 1927, although there is evidence that the trouble has existed in certain isolated areas in Utah for a number of years prior to this date.

The disease may develop suddenly over a vast area in any one season and is capable of extensive and frequently complete destruction in both the early and the late potato crop.

Studies since 1927 have shown the dangerous nature of psyllid yellows and indicate that in certain districts it is a perennial menace even to the extent of eliminating the potato as a crop. This condition exists particularly in Washington County in southern Utah, and also in the early potato-growing areas of Davis and Weber Counties in the northern part of the State. A similar condition is reported also for the Fruita section in the Grand Junction district of Colorado (western slope).

In survey studies the tomato psyllid (*Paratrioza cockerelli* Sulc) has been found constantly associated with psyllid yellows of the potato, and experiments have shown that the disease is in some way induced during the feeding processes of the nymphs of this insect.

Under conditions especially favorable for symptom expression as few as three to five nymphs might occasionally produce psyllid yellows, although uniformity of appearance and full expression of symptoms seldom result with fewer than 15 to 30 actively feeding nymphs.

The adult form of *Paratrioza cockerelli* in numbers up to 1,000 per potato plant appear incapable of producing psyllid yellows symptoms on the plant in the field or in the greenhouse.

The symptomatology of psyllid yellows varies greatly with the number of insects feeding, the length of feeding period, and the intensity and duration of light exposure during the time of feeding.

Psyllid-yellows symptoms under conditions of unmodified sunlight consist of yellowing, basal leaf rolling and purpling of the younger leaves, yellowing and rolling of older leaves, nodal enlargement, increased axillary angle, aerial tubers and shoots, frequent rosetting, various apical growths, and distortion, excess tuberization, and inhibition of rest period. Under conditions of decreased exposure and intensity of light, basal, marginal, and interveinal yellowing becomes a constant feature of the disease.

Progress in symptomatological expression is stopped abruptly when insects are removed at intervals of 12, 16, and 26 days after feeding has commenced, indicating that the full expression of symptoms of psyllid yellows results only when nymphs of *Paratrioza cockerelli* are allowed to feed continuously on the tissues of the infested plant. A tendency toward recovery results when time of feeding in the greenhouse is less than 16 days. Recovery in the field has also been observed. So far as is known, *P. cockerelli* is the only factor capable of producing psyllid yellows in the potato and in related plants.

Attempts to transmit psyllid yellows from diseased to healthy plants have failed. Under Utah conditions the disease does not appear to be transmitted from diseased plants to the following generation through the tuber.

The size, motility, prolific fecundity, longevity, and long oviposition period of the female are important factors in the rate of dissemination of the insect. These factors, when considered in connection with the apparent general distribution of the insect, provide, in part, at least, an explanation of the sudden widespread epidemic of psyllid yellows in 1927.

In preliminary tests nymphs of *Paratrioza cockerelli* were not separated from the infective principle by hatching eggs on healthy potato leaves in Petri dishes. In fact, nymphs so hatched produce a more vigorous symptom response on healthy Irish Cobblers than psyllid nymphs of the same age grown on infected potato plants.

The true nature of the infective principle injected into potato plants by *Paratrioza cockerelli* at present remains unknown. Available facts, however, question somewhat the virus theory of the disease and suggest the possible existence of some toxic substance which is produced in some way during the feeding process of the psyllid nymphs. Additional facts will be necessary before final conclusions can be drawn as to the true etiology of psyllid yellows.

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CHROMOSOMES IN GOSSYPIUM AND RELATED GENERA[1]

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INTRODUCTION

An investigation of the chromosomes in the Malvaceae, particularly in Gossypium, was undertaken because of the repeated failures of cotton breeders in their attempts to hybridize such cottons as Garo Hill (*Gossypium cernuum* Tod.) with any of the varieties of upland cotton (*G. hirsutum* L.).

In 1923 a study was made of the chromosomes in a few representative species of Gossypium growing in the greenhouses at Washington, D. C. Among these were found some species with 13 and some with 26 as their haploid chromosome number. Neither number corresponded with that found earlier by Cannon (*4*)[3] or Balls (*1*). Simultaneously with two other investigators, Denham (*5*, *6*), of England, and Nikoljeva as reported by Zaitzev (*15*, *16*), of Russia, the writer found that cultivated varieties of Gossypium fall into two classes, those with 13 chromosomes and those with 26 chromosomes. This classification corresponds with the taxonomic separation of Gossypium species into the Old World, or Asiatic, and the New World, or American, groups.

MATERIAL AND METHODS

The present investigation has been confined to pollen mother cells during the reduction phases. Young buds were selected and the staminal column removed and put at once into Bouin's, Carnoy's, or chromo-acetic (1:1:100) killing solution, embedded, sectioned, and stained. It was soon found that very satisfactory preparations of pollen mother cells in which the chromosomes were dividing could be made from fresh material stained in aceto-carmine fluid. This method was used whenever growing material was near enough to the laboratory for smear preparations to be made promptly after the young buds were picked. When this was impossible, the anthers were put immediately into killing fluid; later they were embedded or, if Carnoy's killing reagent had been used, it was often possible to make aceto-carmine preparations that served for chromosome studies.

CHROMOSOMES IN THE ASIATIC GROUP OF COTTONS

The term "Asiatic cottons," as now understood, designates a distinct group of Gossypium species having the haploid chromosome number 13, characteristic of all cottons of Asiatic origin thus far examined.

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[2] The writer acknowledges the helpful cooperation of the Divisions of Cotton, Rubber, and Other Tropical Plants and of Egyptian Cotton Breeding, Bureau of Plant Industry. The Asiatic×American F_1 plant used in this study was obtained from J. B. Norton and resulted from an accidental cross in the cotton-breeding nursery at Hartsville, S. C.

[3] Reference is made by number (italic) to Literature Cited, p. 226.

Table 1 shows the Gossypium species and varieties, most of which are endemic to the Old World, in which various investigators have found the basic chromosome number 13.

TABLE 1.—*Cottons (Gossypium spp.) in which the haploid chromosome number 13 was found by various investigators*

Denham (*6*)	Zaitzev (*15, 16*)	Banerji (*2*)	Longley
G. arboreum (3 varieties). G. cernuum. G. indicum (2 varieties). G. neglectum.	G. arboreum. G. herbaceum. G. indicum. G. obtusifolium.	G. arboreum (2 varieties). G. cernuum. G. herbaceum (10 varieties). G. indicum (5 varieties). G. neglectum (9 varieties. G. obtusifolium (2 varieties. G. stocksi.	G. arboreum (C. B.[a] 620). G. cernuum (C. B. 422). G. davidsoni [b] (C. B. 101). G. harknessi (C. B. 862). G. herbaceum (C. B. 254-b). G. indicum (C. B. 430, 472, 517, 726, 727, 743; S. P. I.[c] 12139, 12141, 12142, 12146, 12149). G. intermedium Tod. (C. B. 619). G. neglectum (C. B. 506). G. obtusifolium (C. B. 435). G. sturti [d] (C. B. 564).

[a] C. B. refers to accession number of the Division of Cotton, Rubber, and Other Tropical Plants.
[b] Harland (*8*) reports 13 as the haploid chromosome number for this species.
[c] S. P. I. refers to serial designation number of the Division of Foreign Plant Introduction.
[d] Harland (*8*) reports 26 as the haploid chromosome number for this species.

Pollen mother cells of Gossypium are large and are characterized by a dense granular zone in the cytoplasm just outside the nucleus. This zone has been noted by all the earlier investigators of the Malvaceae and, according to Beal (*3*), it is of importance in the formation of spindle fiber.

During the reduction phases the chromosomes appear unusually small in comparison with the large mother cell. They are frequently well separated, however, and stand out sharply in the comparatively clear nuclear region. Figure 1, A to E, shows the chromosomes in a few representatives of the Asiatic group of Gossypium. A is a diakinesis from *Gossypium herbaceum*. The chromosomes at this stage show variations in shape and their bivalent nature is quite apparent. The two parts of one chromosome at the left have been separated by a slight pressure on the cell. In Figure 2, A and B, are two photomicrographs of the first-division metaphase showing the chromosomes on the plate in *G. cernuum*, variety Garo Hill. Figure 1, B, shows an early first-division metaphase from *G. indicum* Lam. The chromosomes are more compact than in Figure 1, A, and are approaching the plate stage. Figure 1, C, shows a late first-division metaphase of *G. obtusifolium* Roxb. Some of the chromosomes have divided, others have not. Figure 1, D, shows an early first-division telophase in *G. indicum*. The two groups of 13 chromosomes were from different planes and the drawing shows them approaching the two poles of the mitotic spindle. Figure 1, E, is a second-division metaphase of *G. neglectum* Tod., showing clearly the 13 chromosomes.

There are three other cottons that have the same chromosome number as the Asiatic group: *Gossypium davidsoni* Watt., of Lower California and Sonora; *G. harknessi* Brandeg., of Lower California; and *G. sturti* F. v. M., of Australia. Harland (*8*) reports 13 as the chromosome number for *G. davidsoni*, but gives 26 as the number for *G. sturti*. Figure 3, A, shows a typical diakinesis of *G. davidsoni*. The number for *G. sturti* has been determined by the writer on two different

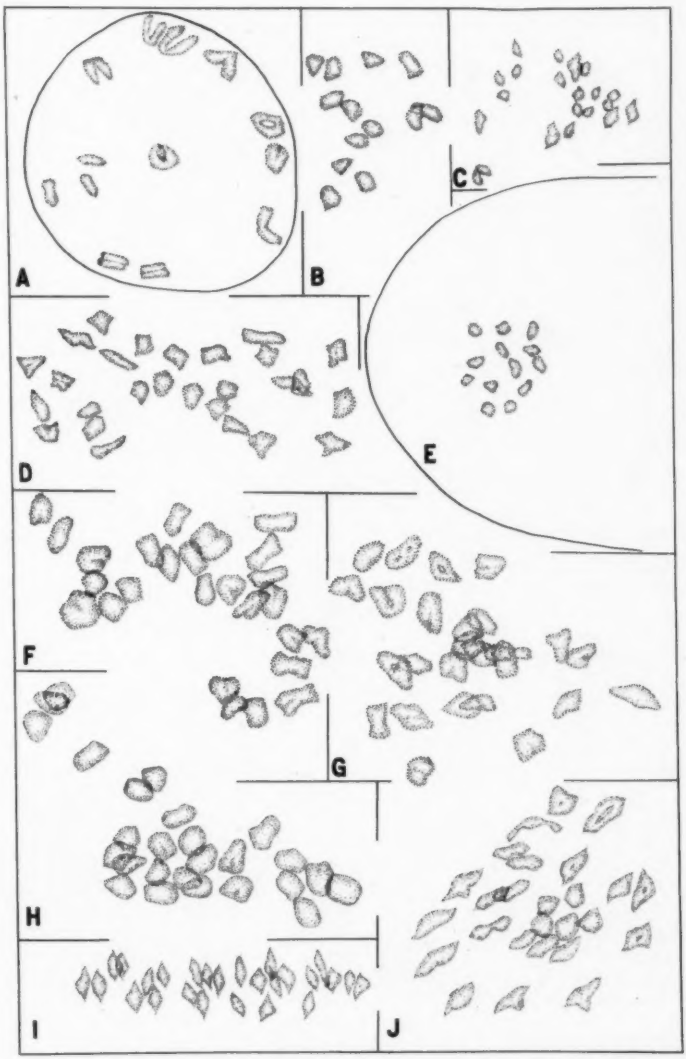

FIGURE 1.—Chromosomes from pollen mother cells of Gossypium (× 1,500): A, Diakinesis of *G. herbaceum;* B, early first-division metaphase of *G. indicum;* C, late first-division metaphase of *G. obtusifolium;* D, early first-division telophase of *G. indicum;* E, second-division metaphase of *G. neglectum;* F, late diakinesis of *G. hirsutum* (Acala); G, late diakinesis of *G. barbadense* (Pima); H, late diakinesis of *G. barbadense* (sea island); I, metaphase of a wild cotton from Florida; J, early metaphase of *G. hirsutum* × *G. barbadense* (Tuxtla × sea island)

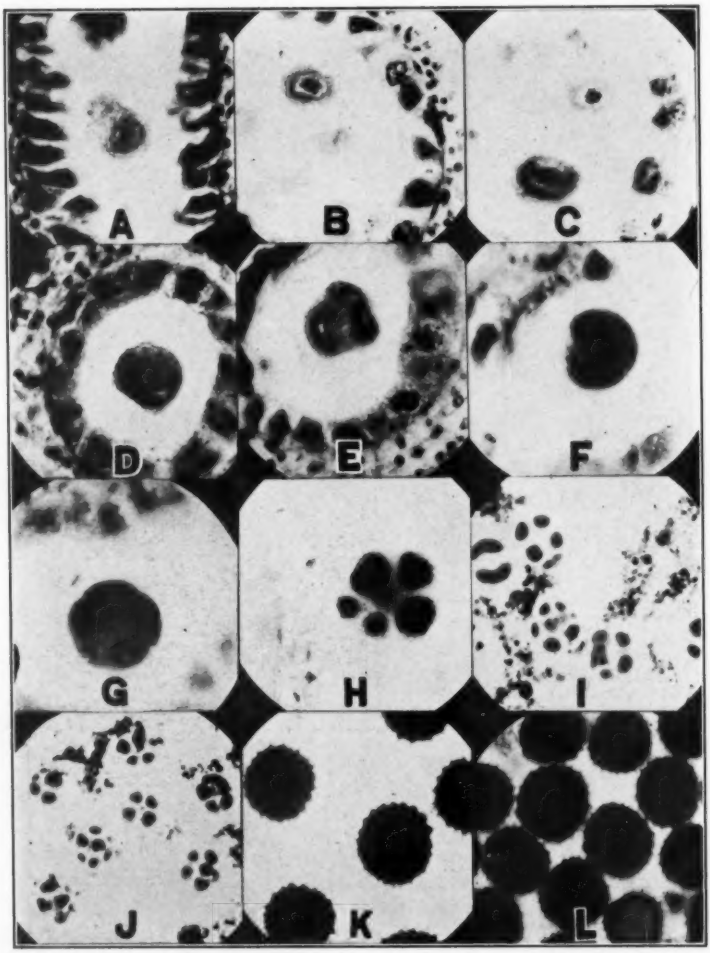

FIGURE 2.—Photomicrographs showing development of pollen in Gossypium (× 500): A and B, First division metaphase of *G. cernuum* (Garo Hill); C, first-division metaphase of *G. hopi* Lewton; D, first-division metaphase of F_1 Asiatic × American hybrid; E, interkinesis of F_1 Asiatic × American hybrid; F, second-division metaphase of F_1 Asiatic × American hybrid; G, late telophase of Asiatic × American hybrid; H, irregular tetrad from Asiatic × American hybrid; I and J, irregular tetrads from F_2 Holdon × Pima (upland × Egyptian) hybrid; K, pollen from sea island (*G. barbadense*); L, pollen from Acala (*G. hirsutum*)

occasions, and in the early phases of the first-reduction division seems to be unquestionably 13.

For *Gossypium stocksi* Mast., a wild species of northwestern India, Youngman and Pande (*14*) found the haploid number 13. This finding has been confirmed recently by Banerji (*2*).

For *Gossypium arboreum* L. × *G. neglectum* and *G. cernuum* × *G. indicum*, two hybrids between species of the Asiatic group, Denham (*6*) found the haploid number 13. He makes no reference to abnormalities in chromosome behavior.

CHROMOSOMES IN THE AMERICAN GROUP OF COTTONS

The American group of cottons is best represented by *Gossypium hirsutum*, typical of the Mexican subgroup, and by *G. barbadense*

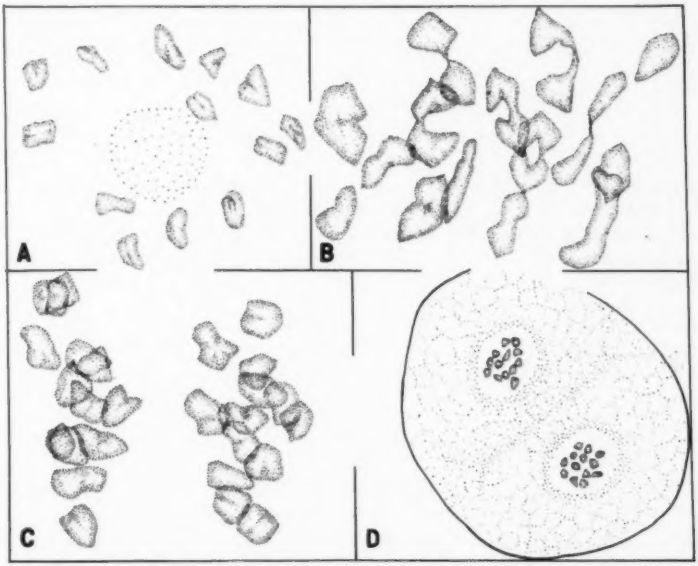

FIGURE 3.—Chromosomes from pollen mother cells of Gossypium and related genera (× 1,500): A, Diakinesis of *G. davidsoni;* B, first-division metaphase of *Shantzia garckeana;* C, first-division anaphase of *S. garckeana;* D, second-division metaphase of *Thurberia thespesioides*

L. and *G. peruvianum* Cav., typical of the South American subgroup. These subgroups apparently include all cottons endemic to North America and South America, except the wild species *G. davidsoni* and *G. harknessi*, of Lower California, and *G. klotzschianum* Anderss., of the Galápagos Islands.

Table 2 shows the Gossypium species and varieties of the Mexican and South American subgroups in which various investigators have found the chromosome number 26.

A late diakinesis of Acala cotton (*Gossypium hirsutum*) is shown in Figure 1, F; in Figure 1, G and H, are shown similar phases of two cultivated forms of *G. barbadense*, Pima and sea island, respectively. In all three cases the 26 chromosomes are shown just before collecting on the plate. Figure 1, I, shows a side view of the first-division meta-

phase plate from a cotton growing wild in Florida. By pressure on the cell the 26 chromosomes were spread out and separated in order to show their characteristic shapes just prior to division. In Figure 2, C, is a photomicrograph showing the chromosomes on the plate in *G. hopi*.

TABLE 2.—*Cottons (Gossypium spp.) in which the haploid chromosome number 26 was found by various investigators*

Denham (*5, 6*)	Zaitzev (*15, 16*)	Banerji (*2*)	Beal (*3*)	Longley
G. barbadense (15 varieties).[a] G. hirsutum (5 varieties).	G. barbadense. G. brasiliense. G. fruticulosum. G. hirsutum. G. lanceolatum. G. mexicanum. G. microcarpum. G. mustelianum. G. palmerii. G. peruvianum. G. punctatum. G. purpurascens. G. schotti. G. vitifolium.	G. hirsutum (3 varieties).	G. barbadense (2 varieties). G. hirsutum (3 varieties).	G. barbadense (C. B. 331-c, 363, sea island and Pima). G. brasiliense (C. B. 535). G. hirsutum (Acala). G. hopi (2 varieties). G. punctatum (C. B. 437).

[a] Balls (*1*) gives 28 as the chromosome number of this species.

In a study of 26 American cottons no exception has been found to the characteristic haploid chromosome number, 26.

Cannon (*4*) was the first to investigate the chromosomes in Gossypium. In his study of a hybrid between *G. barbadense* and *G. hirsutum*, representing a cross between two distinct American types, he found 20 as the haploid chromosome number. Figure 1, J, shows the chromosomes in a similar cross, between the varieties Tuxtla (*G. hirsutum*) and sea island (*G. barbadense*). This hybrid had 26 as its haploid chromosome number, and shows no marked abnormalities in chromosome behavior.

CHROMOSOMES IN A CROSS BETWEEN A PLANT OF THE ASIATIC GROUP AND ONE OF THE AMERICAN GROUP

Recently Zaitzev (*15, 16*) and Desai (*7*) have described successful crosses made between plants of the Asiatic and American groups of cotton. Their attempts to hybridize plants of the Asiatic and American groups gave a large percentage of failures, but a few F_1 plants were obtained. The pollen of these plants proved to be sterile. An Asiatic $\times$American F_1 plant studied by the writer produced abundant flowers, but like those of Zaitzev and Desai, has been perfectly sterile.

Pollen mother tissue was sufficiently abundant in the anthers to make possible a detailed examination of the chromosomes during the reduction phases. Figure 4, C, shows a characteristic first-division metaphase with 14 bivalent and 11 univalent chromosomes. The number of paired chromosomes was variable; only when they could be distinguished from the univalent chromosomes was it possible to get accurate counts. Figure 2, D, shows a photomicrograph of a typical first-division spindle, with the bivalents on the plate and the univalents scattered on the spindle.

In Figure 2, E, is a photomicrograph taken at interkinesis, showing two major nuclei and a minor nucleus. This photograph also gives a fair idea of the granular zone in the cytoplasm that surrounds the nuclei.

A view of the second-division metaphase in a pollen mother cell is shown in Figure 4, D. One plate has 20 full-sized chromosomes and 2 halves of a univalent chromosome that divided in the previous division. The other plate shows 17 large chromosomes and 2 halves of a divided univalent. A much more irregular and more typical second-division metaphase is shown in Figure 4, E. Six spindles have been formed. The chromosome number in each spindle varies from a single univalent to 13 univalents and 6 halves of divided univalents.

Figure 2, F, shows a photomicrograph of a pollen mother cell with two major spindles and a small spindle on the margin of the cell.

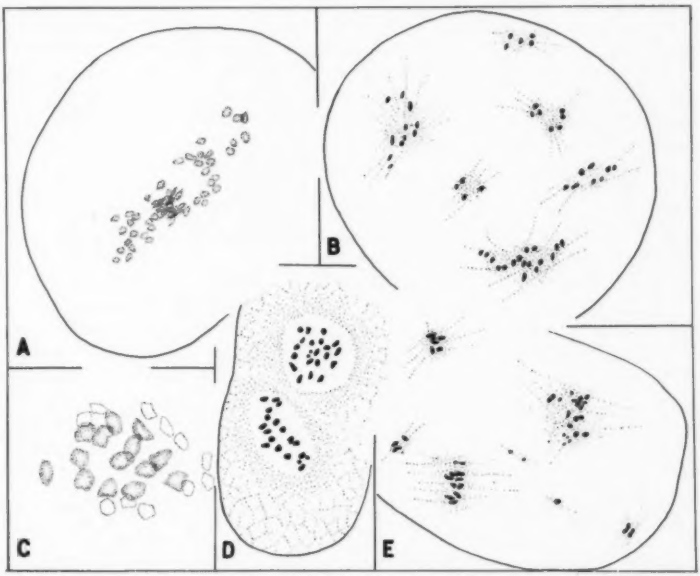

FIGURE 4.—Chromosomes from pollen mother cells of Gossypium hybrids: A, First-division metaphase of an F_2 plant of Holdon ×Pima (upland×Egyptian); B, second-division metaphase, showing 6 spindles of an F_2 plant of Holdon ×Pima; C, diakinesis of an Asiatic ×American hybrid, showing bivalents and univalents in outline; D, second-division metaphase of an Asiatic×American hybrid, showing unequal numbers of chromosomes on the two plates; E, second-division metaphase of an Asiatic ×American hybrid, showing 2 major and 5 minor spindles

Figure 2, G, shows the late telophase of a pollen mother cell just prior to cell division.

Chromosome distribution during the reduction phases of the hybrid was generally irregular and led to the production of abundant abnormal pollen tetrads and sterile pollen.

Nakatomi (*10*) has recently described the cytological behavior of the chromosomes during pollen formation in an American × Asiatic F_1 hybrid. In this cross, although it differed in some details from the reciprocal cross described by the writer, Nakatomi found the pollen tetrads similar and the mature pollen variable in size and apparently sterile.

CHROMOSOMES IN RELATED GENERA

A study was likewise made of the chromosomes in certain genera closely allied to Gossypium.

For *Thurberia thespesioides* A. Gray, the haploid chromosome number proved to be 13, the same as that of the Old World species of Gossypium. Figure 3, D, shows a typical pollen mother cell from this species, in which 13 chromosomes show distinctly in the two metaphase plates.

Shantzia garckeana Lewton, another plant related to Gossypium, was grown in the greenhouses at Washington, D. C., from seeds collected by H. L. Shantz in Central Africa. This species also has 13 as its haploid chromosome number. Figure 3, B and C, shows the chromosomes of Shantzia during a late first-division metaphase in which 2 of the chromosomes have divided, and a first-division anaphase in which 13 chromosomes are approaching each pole.

For *Sidalcea neomexicana* A. Gray, of the tribe Malveae, Tjebbes (*12*) found the haploid chromosome number 13, the basic chromosome

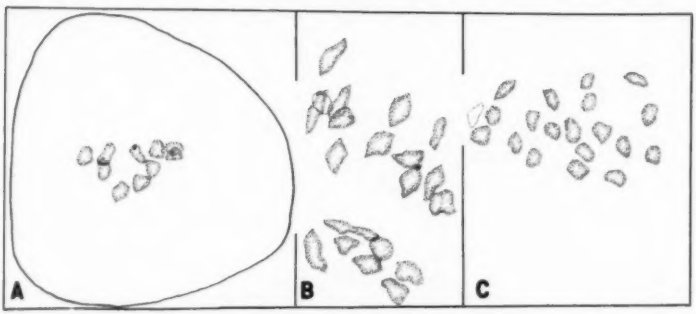

FIGURE 5.—Chromosomes from pollen mother cells of Gossypium relatives (× 1,500): A, Early first-division metaphase of *Cienfugosia heterophylla;* B, early first-division metaphase of *Hibiscus militaris;* C, early first-division metaphase of *H. palustris*

number of the genera Gossypium, Thurberia, and Shantzia, of the tribe Hibisceae.

Figure 5, A to C, shows the chromosomes in three other representatives of the tribe Hibisceae. *Cienfugosia heterophylla* (Vent.) Garcke, a species native to the southern part of the United States, has 10 bivalent chromosomes as shown in a first-division metaphase. (Fig. 5, A.)

Figure 5, B and C, shows the chromosomes from pollen mother cells of two representative Hibiscus species, *H. militaris* Cav. and *H. palustris* L. Counts were unusually difficult to make. Although 19 seemed to be the haploid chromosome number for both species, each figure shows a shadowy outline of what may be a twentieth chromosome. Youngman (*13*) studied the chromosomes of the species *H. rosa-sinensis* L., *H. tricuspes* Banks, and *H. tiliaceus* L. He experienced considerable difficulty in making counts, but from his best preparations he gives the diploid chromosome numbers 72, 40, and 48 for the three species, respectively.

For *Lavatera thuringiaca* L. and *Malva moschata* L., of the tribe Malveae, Svensson-Stenar (*11*) found the haploid chromosome num-

ber 20. He gives the haploid chromosome number of *Malva palmata* Hedl. as approximately 20 and that of *M. pusilla* Sm. as between 20 and 30. These numbers are in harmony with the numbers found for Cienfugosia and Hibiscus of the tribe Hibisceae. The chromosome number 21 for *Malvastrum capense* Garcke, however, must be an aneuploid representative if it belongs to any of the chromosome series already described for Malvaceae.

POLLEN STERILITY

In conjunction with chromosome studies and variations in the reduction phases of the pollen mother cell, the pollen tetrad often is of considerable interest. Normally there are four equal-sized daughter cells in the tetrad. A deviation in the size or number of daughter cells represents an abnormality. If there is a variation in the size of the cells it is generally attributed to an unequal distribution of the chromosomes in either the first or second division. The presence of more than four cells shows not only that there has been an unequal distribution of the chromosomes but also that some chromosomes have failed to be included in the 2-daughter nuclei of the first division or in the 4-daughter nuclei of the second division. These extruded chromosomes develop into micronuclei and small pollen grains.

As a rule, cottons have only a small percentage of abnormal tetrads and the mature pollen looks uniform in size and shows only a very few sterile grains. In plants with a large percentage of sterile pollen a considerable number of tetrads were found to be abnormal. In a crinkly-leaf variant of the Pima variety (*Gossypium barbadense?*), for example, 15 per cent of the tetrads were abnormal and approximately 50 per cent of the pollen sterile. Some off-type plants, however, showed little sterile pollen. One plant, although showing considerable sterile pollen, had apparently normal tetrads. Abnormalities in the tetrads of a plant show that the chromosome behavior during pollen formation has been abnormal, and in such a plant there will be an appreciable amount of pollen sterility. The presence of sterile pollen in a plant, however, does not always indicate abnormal chromosome distribution and abnormal tetrads, for in the rogue mentioned above and in occasional plants in F_2 progenies of Acala $\times$ Pima hybrids, considerable sterile pollen was found that could not be traced to abnormalities in the chromosome distribution at the time of pollen formation.

SUMMARY AND CONCLUSIONS

Recent studies of the chromosome numbers in Gossypium made by the writer and by other investigators show the existence of two distinct groups, namely, the Asiatic group, in which the haploid chromosome number is 13, and the American group, in which the haploid chromosome number is 26. The separation of Gossypium species into two groups based on chromosome number corresponds with the general division of the species based on taxonomic characters.

This classification applies to the cultivated cottons and does not take into account certain wild species of Gossypium. Two of these, *Gossypium davidsoni* and *G. harknessi*, although indigenous to North America, have 13 chromosomes, the number characteristic of Old World cottons.

It is customary to consider the diploid representatives of a group of plants as being more nearly related to the ancestral type than those forms having higher chromosome numbers. Five species, so isolated that there has been little opportunity for contamination with their close relatives, may be considered as representing most nearly the ancestral type. These are *Gossypium stocksi*, *G. sturti*, *G. davidsoni*, *G. harknessi*, and *Thurberia thespesioides*. The last, although usually considered as a separate genus, is very nearly related to Gossypium. All these species grow in more or less isolated localities and hence may represent the primitive stocks from which the cultivated varieties of cotton have arisen. The fact that they have only 13 chromosomes indicates that 13 was the haploid and 26 the diploid chromosome number of the ancestors of our present-day cottons.

The double chromosome number found in the American cottons suggests a duplication of the chromosomes of an ancestral type. Cytologically this has not been demonstrated. If Gossypium species having 26 chromosomes are true tetraploids, simple Mendelian ratios should appear much less frequently in them than in the Asiatic group. Matsuura (*9*) has brought together the studies on inheritance in Gossypium. These show that about half of the characters studied in the American group segregate in a 3:1 ratio, while in the Asiatic group all but two characters studied are distributed in a 3:1 ratio. Such a method of comparison is not conclusive evidence of the duplication of chromosomes in all species with 26 chromosomes, but it at least suggests that the American species of Gossypium are tetraploid.

Many of our American cottons have had ample opportunity to cross with each other. It is possible that their high chromosome number has arisen through hybridization and that this new stock of vigorous hybrids has obliterated many of the ancestral types. If American cottons are both polyploid and crypt hybrids, it is to be expected that they will show the same difficulties of taxonomic classification encountered in many polymorphic genera, in which intermediate forms often bridge the gap between distinguishing specific characters.

Of the other genera of the Malvaceae investigated, only Thurberia and Shantzia were found to have chromosome numbers suggesting a close relationship to Gossypium. The numbers in other Malvaceae are, however, distinctive, and a critical study of representatives of other genera may lead to the discovery of chromosome numbers indicating relationships.

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THE VITAMIN A CONTENT OF ALFALFA AS AFFECTED BY EXPOSURE TO SUNSHINE IN THE CURING PROCESS[1]

By MARGARET CAMMACK SMITH, *Nutrition Chemist*, and IAN A. BRIGGS, *Assistant Agronomist, Arizona Argicultural Experiment Station*

INTRODUCTION

Experiments in this laboratory have shown the value of alfalfa as a source of vitamin A in animal rations composed largely of the sorghum grain Hegari (*6*)[2]. In 1925 Steenbock and his coworkers (*10*) reported a loss in vitamin A accompanying the bleaching of clover hay due to excessive exposure to sunlight. The association of vitamin A with carotinoid pigment was pointed out at that time and since then considerable evidence showing the similarity in distribution of vitamin A and yellow or green coloring material has been accumulated (*7, 8, 9, 11*). More recently Moore (*1, 2*) has proved rather conclusively that carotene in plants is converted into vitamin A in the animal body and perhaps can be considered the precursor of vitamin A as ergosterol is the precursor of vitamin D. The inferior vitamin A value of discolored, bleached alfalfa is therefore to be expected.

It was the purpose of the work described in this paper to measure the comparative destructive effect of curing periods of different length on the vitamin A content of alfalfa produced in Arizona. Since this work was begun, Russell (*3*) has reported that machine-dried alfalfa contained about seven times as much vitamin A as that cured on the field in the usual manner in New Jersey.

EXPERIMENTAL PROCEDURE

PREPARATION OF THE ALFALFA

The alfalfa used was produced on the Salt River Valley farm at Mesa, Ariz. It was of the Hairy Peruvian variety, and of the third cutting from a planting in its second crop year. The alfalfa was growing thriftily, the plants were about 22 inches high, and the stand was thick. The soil on which the crop was produced is classified as Maricopa silt loam and definitely is alkaline in reaction.

The hay was cut with a mower at 11.15 a. m., June 24, 1929, that used in the experiment all being cut within the space of a few minutes. Immediately after the hay was cut, portions were spread out on the surface of the swaths so that the individual stalks were separated and did not overlap. This was done to secure maximum exposure to the sun. The spreading was done by four men and approximately one hour was required for the work. Care was taken to use only the stalks from the portions of swath most exposed to the sunlight after the first few minutes.

One sample was taken to a darkened curing house immediately after it was cut. All samples were placed on racks in this curing shed as soon as removed from the field and curing was completed in the shade in the absence of either sunlight or daylight. Windows

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[2] Reference is made by number (italic) to Literature Cited, p. 233.

were darkened so that very little light reached the curing hay. Ventilation was provided so that the hay dried without spoilage or discoloration.

The samples of alfalfa were removed from the field as shown in Table 1.

TABLE 1.—*Time of removing alfalfa, cut at 11.15 a. m., June 24, 1929, and period during which it remained in the field*

Sample No.	Date and hour removed from the field	Hours in the field	Sample No.	Date and hour removed from the field	Hours in the field
1	June 24, 11.15 a. m	0	4	June 24, 6 p. m	6¾
2	June 24, 2 p. m	2¾	5	June 25, 8 a. m	20¾
3	June 24, 4 p. m	4¾	6	June 25, 12 m	24¾

The day on which the hay was cut and the following day were classed as "clear" in the weather records. There was a slight haze the first of the two days which may have interfered with the intensity of the rays of the sun to some extent. The temperature was higher on the day the alfalfa was cut than on any other day of that year. A temperature of 115° F. was recorded at noon of that day. The meteorological data for the two days are shown in Table 2.

TABLE 2.—*Meterological data for days on which alfalfa samples were cut*

Day	Air temperature		Wind movement	Evaporation
	Maximum	Minimum		
	°F.	°F.	*Miles per hour*	*Inch*
June 24	115	66	20	0.444
June 25	110	71	21	.372

Only the leaves of the alfalfa were used in the laboratory experimental work so as to insure more uniform samples. The leaves were carefully separated from the stems and petioles by hand, finely ground, and kept in covered glass jars in the laboratory until used.

METHOD OF MEASUREMENT OF VITAMIN A CONTENT

The method of measuring the vitamin A content of the different samples of alfalfa allowed to lie on the field for different periods of time is that developed by Sherman and Munsell (*5*) and modified somewhat by Sherman and Burtis (*4*). Albino rats taken from Sherman's diet B at the time of weaning were placed on a vitamin-A-free ration composed of 67 per cent cornstarch, 10 per cent dried yeast, 18 per cent alcohol-extracted casein, 4 per cent Osborne and Mendel's salt mixture, and 1 per cent sodium chloride. Vitamin D was provided by the incorporation of 0.5 g [3] of irradiated cholesterol in each 1,000 g of diet. When the store of vitamin A present in the bodies of the rats at weaning was exhausted, they were placed in individual round metal cages with screen bottoms and given the basal diet and distilled water ad libitum. Weighed amounts of the

[3] g is the abbreviation for gram or grams.

ground alfalfa leaves to be tested were fed daily for an experimental period of eight weeks. Record was kept of the weekly weights and general condition of each animal. At least two representative animals from each litter were kept as negative controls. At the end of the test period the animals still surviving were chloroformed, autopsy observations of such evidences of vitamin A deficiency as infection in glands at the base of the tongue, eyes, ears, sinuses, bladder, etc., were noted. These findings, in conjunction with the growth response to the alfalfa supplements, were used as criteria of the comparative amounts of vitamin A in the different alfalfa samples fed.

EXPERIMENTAL DATA AND DISCUSSION OF RESULTS

The summarized results of measurement of the vitamin A values of samples of alfalfa which have been cured on the field for different lengths of time appear in Table 3.

TABLE 3.—*Summarized results of feeding alfalfa leaves, differently treated in the field, to albino rats as the sole source of vitamin A*

Sample No.	Field treatment of alfalfa	Rats used	Average weight at end of fore-period	Alfalfa fed daily	Average gain (+) or loss (−) of rats in 8-week test period	Health and autopsy observations
		Number	*Grams*	*Grams*	*Grams*	
1	Removed as soon as cut and cured in the dark	6	125.0	0.005	+22.3	Slight infection in 2 cases.
		8	122.0	.0075	+41.8	No symptoms of vitamin A deficiency.
		10	134.7	.01	+61.7	Do.
2	Spread for 2¾ hours	2	122.6	.005	(a)	Severe infections in all cases.
		8	125.0	.0075	+19.0	Some infection in 3 cases.
		8	130.2	.01	+36.3	No symptoms of vitamin A deficiency.
3	Spread for 4¾ hours	2	118.0	.005	−1.8	Severe infections in all cases.
		8	123.6	.0075	+17.0	Some infection in 4 cases.
		8	127.0	.01	+39.5	No symptoms of vitamin A deficiency.
4	Spread for 6¾ hours	2	119.6	.005	+9.0	Severe infections in both cases.
		8	122.5	.0075	+24.1	Slight infections in 3 cases.
		8	117.3	.01	+35.0	No symptoms of vitamin A deficiency.
5	Spread for 20¾ hours	4	129.0	.0075	(a)	Severe infections in all cases.
		8	122.0	.01	+2.8	Severe infections in 6 cases.
		8	119.0	.02	+18.0	Severe infections in 4 cases.
		6	125.6	.03	+39.5	No symptoms of vitamin A deficiency.
		3	124.0	.0075	(a)	Severe infections in all cases.
		8	126.2	.01	(a)	Do.
6	Spread for 24¾ hours	6	126.5	.02	−9.2	Do.
		6	119.3	.03	+17.0	Some infections in all cases.
		2	131.2	.05	+49.0	No symptoms of vitamin A deficiency.
7	On field in swath for 1 week	2	100.0	1.02	(a)	Severe infections in all cases.
		4	107.0	.05	(a)	Do.
		6	106.0	.1	−8.0	Do.
		6	112.0	.2	+36.?	No symptoms of vitamin A deficiency.
8	Negative controls	25	122.6	0	(a)	Severe infections in all cases.

[a] Died before end of test period.

Alfalfa which was removed from the field as soon as cut and dried in a darkened house is seen to be a very excellent source of vitamin A. Five milligrams of these alfalfa leaves fed daily were sufficient to induce only slightly less gain than 3 g a week during the 8-week test period, which is Sherman's vitamin A unit gain. A gain of 41.8 g and 61.7 g in the test period, or of approximately 5 and 8 g per week, resulted from the daily feeding of 7.5 and 10 mg, respectively, of the alfalfa leaves prepared in the same fashion.

When, however, the alfalfa was spread out in the field for periods ranging in length from 2¾ to 6¾ hours 5 mg of the leaves failed to provide enough vitamin A to permit even the low rate of gain of 3 g a week. The animals dependent upon these samples of alfalfa fed at this low level for their vitamin A were not protected against the severe infections characteristic of vitamin A deficiency. All of the animals in this group showed signs of ophthalmia varying in degree from + to + + + + before death, and autopsies revealed infection in glands at the base of the tongue, ears, and sinuses. Increasing the quantity of alfalfa fed to 7.5 mg daily provided sufficient vitamin A to induce slightly less than the unit rate of gain (17–24 g in eight weeks). Only slight infections of eyes and sinuses were observed in these groups of animals. Again when the quantity of these three samples of alfalfa fed daily was further increased to 10 mg, not only was an average gain of from 35 to 39.5 g promoted in the three groups of animals, but all of the animals were free from the infections usually accompanying a marked vitamin A deficiency.

None of these samples of alfalfa had suffered loss of green color indicative of bleaching, and observable to the naked eye, as a result of their exposures on the field. In spite of this fact it is obvious that some destruction of vitamin A did result during this period, because it required the daily feeding of from one-fourth to one-half as much again to induce the same rate of gain in the test animals as was obtained by the feeding of the alfalfa cured in the dark. These alfalfa leaves which were exposed to the sun over the noon hour, 2¾ hours, therefore, contained from 20 to 33 per cent less vitamin A than those which had been removed from the field immediately after cutting and cured in the dark. However, no significant difference in vitamin A content resulting from 2¾, 4¾, and 6¾ hours of exposure was detected in these experiments.

When the alfalfa was allowed to lie on the field overnight, 11.15 a. m. one day until 8 a. m. the next, or a period of 20¾ hours, a decidedly greater degree of vitamin A destruction, again basing the comparison on the vitamin A content of the cured in the dark sample, was observed. In this case, even 10 mg daily failed to induce gains or protect the test animals from infections attributable to vitamin A deficiency. Twenty milligrams of the material was necessary approximately to produce the vitamin A unit gain in weight, and 30 mg were necessary to induce a growth of 39.5 g in the experimental period. These results are indicative of approximately 75 per cent destruction as a result of this period of exposure.

With an additional four hours of exposure to morning sunshine on the second day, the destruction was even greater. Roughly only 16 per cent of the vitamin A present in the sample cured in the dark was retained in the alfalfa dried in the dark after being spread out in the field for 24¾ hours. That both the 20¾-hour and the 24¾-hour samples had suffered some bleaching was readily observed, especially in the stems.

The rate of destruction is more rapid at first and then decreases as the time of exposure is extended. Alfalfa exposed in the swath for one week, during which time it received 57 hours of sunshine and 0.37 inch of rain, was severely bleached and retained only approximately 4 per cent of the vitamin A found in the sample cured in the dark.

These experiments supply further evidence that the conditions of the curing process bear a marked relation to the vitamin A content of alfalfa hay. In order to retain maximum vitamin A potency and thus best to preserve its nutritional value, it is evident that exposure to Arizona sunshine should be reduced to a minimum, and the alfalfa cured as quickly as possible to inhibit destructive oxidative or enzymatic changes.

SUMMARY

The vitamin A value of alfalfa leaves which have been allowed to lie spread out in the field for varying lengths of time has been compared with the vitamin A content of alfalfa leaves taken from the field immediately upon cutting and dried in a well-ventilated darkened house.

A loss of from 20 to 33 per cent of the vitamin A found in the sample cured in the dark resulted from allowing the alfalfa to lie carefully spread out on the field for the 2¾-hour period from 11.15 a. m. to 2 p. m. No greater degree of destruction was observed when the time of exposure was increased to 6¾ hours in the same day. No loss of green color in these alfalfa leaves could be observed.

A 75 per cent loss of vitamin A occurred when the alfalfa was allowed to lie on the field under the same conditions overnight, and until 8 a. m. the next day. This loss was increased to approximately 84 per cent upon a further 4-hour period of exposure until 12 noon the second day. These alfalfa leaves retained a large part of their green color, although the stems showed evidence of marked bleaching, but only 16 per cent of the vitamin A present in the sample cured in the dark was retained.

Alfalfa severely bleached as a result of 1-week exposure to sun and rain on the field in the swath retained but 4 per cent of the vitamin A present in the sample cured in the dark.

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THE ANTIRACHITIC VALUE OF ALFALFA AS AFFECTED BY EXPOSURE TO SUNSHINE IN THE CURING PROCESS [1]

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INTRODUCTION

Alfalfa, a roughage widely used in animal rations, is now valued for its vitamin A content and calcifying properties.

The antirachitic properties of hays were first demonstrated to be related to their exposure to sunshine by Steenbock et al. in 1925 (*7*).[2] They showed that clover hay when made in the sunlight possessed calcifying properties which hay cured in the dark did not have. As Arizona receives a high percentage of the total possible sunshine all the year round, it seemed desirable to investigate the vitamin D content of Arizona-grown alfalfa, and to study the relation between curing periods of different lengths, under field conditions, and the calcifying properties of the hay.

Since this work was begun, Russell (*4*) of the New Jersey Agricultural Experiment Station has reported that artificially cured alfalfa leaves contained only small quantities of the antirachitic vitamin, but when alfalfa was dried in the sun without exposure to dew or rain there was an increase in the antirachitic potency of the leaves. This is in agreement with the findings of Steenbock and his coworkers.

EXPERIMENTAL PROCEDURE

MATERIALS USED

The alfalfa used in this study was prepared as described in detail in a previous paper (*5*). It was originally planned to measure the antirachitic value of all the samples used in the vitamin A study, but the early results obtained did not justify such an intensive study from the practical standpoint. Accordingly results reported in this paper deal with only three samples of alfalfa.

Sample No. 1 was taken from the field as soon as cut, 11.15 a. m., July 8, 1930, and cured in a dark well-ventilated room.

Sample No. 2 was spread out by hand and allowed to lie on the field from 11.15 a. m. one day (July 8) to 12 noon the next day, during which time it received 15 hours and 15 minutes of exposure to sunshine of varying intensity.

Sample No. 3 was allowed to lie on the field in the swath for one week. The maximum temperature during this period ranged from 93° to 104° F., the minimum temperature from 67° to 79°. During this 1-week period the sun shone 57.3 hours, but the alfalfa received only 42 per cent of the possible sunshine, for several of the days were cloudy and there was 0.37 inch of rainfall.

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[2] Reference is made by number (italic) to Literature Cited, p. 240.

In order to insure uniformity of sampling only the leaves of the alfalfa were used, as was the case in the previous experiments. The leaves were carefully separated from the stems by hand and finely ground.

MEASUREMENT OF THE ANTIRACHITIC POTENCY

The rickets curative method, essentially that suggested by the work of McCollum (*3*) and his coworkers, was used to measure the comparative antirachitic value of alfalfa samples cured for different lengths of time. Albino rats weighing from 55 to 64 g [3] were taken from the stock colony at the time of weaning and were placed upon Steenbock's (*6*) high calcium-low phosphorus rachitic ration No. 6529, composed of 76 per cent ground yellow corn, 20 per cent wheat gluten, 3 per cent calcium carbonate, and 1 per cent sodium chloride.

The rats, when kept in the dark, regularly become rachitic on this ration in 21 days, as evidenced by their enlarged joints and rachitic gaits. At this time a Shipley (*3*) "line test" on the proximal end of the tibia of a representative animal of each litter showed a wide metaphysis typical of severe rickets.

The remaining rats in each litter were then placed in individual metal cages with raised screen bottoms and given distilled water ad libitum. As some difficulty was encountered in getting the rats to eat quantitatively the ground alfalfa when fed separately, it was incorporated in the ration at a level of 5 and 25 per cent, replacing equivalent amounts of yellow corn. That the high calcium-low phosphorus ratio was not seriously disturbed was shown by analyses of the resulting rations showing a Ca and P ratio varying from 3.7 to 1 in diet 2965 to 4.7 to 1 in an experimental ration containing 25 per cent ground dried alfalfa leaves.

A record was kept of the food intake and daily gains in weight of each rat during the experimental test periods, and those animals refusing food and not gaining in weight were discarded. Several representative rats from each litter were kept upon the unsupplemented rickets-producing ration No. 2965 as negative controls, one control always being retained until the last rat in each litter had been chloroformed, its tibia dissected free from flesh and tested for rachitic lesions by means of the line test. If evidence of the so-called spontaneous healing of the rachitic lesions of the control animals was obtained, its litter mates were discarded. In the early fall some difficulty was experienced in the production of rickets in the usual 21-day period and at the same time, unexplainable cures of rickets in the control animals on the basal rachitic rations occurred. The recent findings of Harris and Bunker (*2*) that freshly ground corn has antirachitic properties which are destroyed during the aging process satisfactorily explain the difficulties encountered, for at the time a supply of the new crop of yellow corn was being used in the ration.

Degree of healing of the rachitic lesions was graded one to four positive (+ to + + + +) according to the method of Bills et al. (*1*).

Table 1 shows the results of feeding to rachitic animals dried ground alfalfa leaves taken from the field as soon as cut and cured in the dark.

[3] g is the abbreviation for gram or grams.

TABLE 1.—*Summarized results of feeding various quantities of alfalfa leaves, cured in the dark, to rachitic rats*

[The line-test findings denoted no healing in any case]

Percentage of alfalfa in the ration	Rats used	Length of test period	Average gain in weight in test period	Average daily food intake
	Number	*Days*	*Grams*	*Grams*
5	2	5	4.0	7.0
	2	7	7.0	6.4
	6	14	12.5	6.4
	2	21	18.0	6.4
	1	28	18.0	8.5
15	2	7	5.0	7.3
25	2	5	4.0	6.8
	6	7	6.3	8.2
	3	14	15.5	8.0
	6	21	21.7	9.0

Obviously the alfalfa leaves that were taken from the field immediately upon cutting and cured in the dark contained no appreciable amounts of the antirachitic vitamin, if any at all. Inclusion in the basal ration of as much as 25 per cent ground alfalfa leaves did not heal the rachitic lesions of the test animals even when fed over a period of three weeks. In no case did a line at the zone of provisional calcification appear when these alfalfa leaves were included in the ration.

As a further test of the absence of vitamin D in alfalfa which had not been exposed to sunlight, 18 rats representing three litters were selected at the time of weaning, half of them placed upon Steenbock's rachitic ration No. 2965, and the other half, under similar conditions, on the same ration in which 25 per cent ground dried cured in dark alfalfa leaves had replaced an equivalent weight of the yellow corn. At the end of 21 days the animals were killed and a line test was made upon the left tibia of each animal. Rickets of equal degree of severity was observed in all of the animals both on diet 2965 and the alfalfa ration, thus showing that 25 per cent alfalfa leaves in the ration neither prevented the development of rickets nor cured rachitic lesions that had already developed. In this manner further evidence of the low antirachitic potency of the alfalfa, when unexposed to sunlight, was obtained.

TABLE 2.—*Summarized results of feeding various quantities of alfalfa leaves, cured for 24¾ hours on the field, to rachitic rats*

Percentage of alfalfa in the ration	Rats used	Length of test period	Average gain in weight	Average daily food intake	Line-test findings
	Number	*Days*	*Grams*	*Grams*	
5	5	5	4.5	5.0	No healing in any case.
	4	7	8.0	6.0	Do.
	7	14	13.8	7.8	No healing in 5 cases, + healing in 2 cases.
	7	21	17.0	8.2	No healing in 5 cases, ++ healing in 2 cases.
	4	28	32.7	9.5	No healing in any case.
25	10	5	4.0	6.2	No healing in 8 cases, ++ healing in 2 cases.
	7	7	2.0	6.7	No healing in 6 cases, ++ healing in 1 case.
	2	10	9.0		++ healing in both cases.
	12	14	11.7	8.3	++ healing in 2 cases, +++ healing in 5 cases, ++++ healing in 5 cases.

Following the same procedure, measurement was made of the antirachitic value of alfalfa sample No. 2 which had remained on the field from 11.15 a. m. one day until 12 noon the next day, a period of 24¾ hours, before being gathered.

The results are presented in Table 2.

From Table 2 it is clear that alfalfa which has been cured on the field for 24¾ hours in Arizona possesses calcifying properties that alfalfa cured without exposure to sun does not have. Even under these conditions the synthesis of the antirachitic vitamin has not been great, for the inclusion of 5 per cent of this alfalfa in the ration resulted in no healing of the rachitic lesions of rats in the majority of cases tested, and in but slight healing in 4 out of 27 cases. Again, it was found possible to produce rickets in rats that had been fed from the time of weaning upon the rickets-producing ration in which 5 per cent of the 24¾-hour-exposure alfalfa leaves were substituted for an equal weight of yellow corn. When these animals were kept in the dark, rickets of a degree only slightly less severe than that of the control animals on diet 2965 developed in the 21-day test period. That the calcifying properties of the alfalfa had been increased, however, by allowing it to lie on the field for 24¾ hours, was made evident by the inclusion of 25 per cent of the leaves in the basal ration. At this higher level of feeding healing was practically complete in a test period of two weeks, although it was not regularly obtained in the 5-day test period advocated by Bills (*1*).

Results of feeding to rachitic rats alfalfa sample No. 3 which had been allowed to lie in the swath for a week appear in Table 3.

TABLE 3.—*Summarized results of feeding various quantities of alfalfa leaves, cured for one week on the field, to rachitic rats*

Percentage of alfalfa in the ration	Rats used	Length of test period	Average gain in weight	Average daily food intake	Line-test findings
	Number	*Days*	*Grams*	*Grams*	
5	4	3	1.0	8.0	++ healing in all cases.
	7	5	4.0	7.4	+++ healing in 2 cases, ++++ healing in 5 cases.
	7	7	5.8	7.0	++++ healing in all cases.
	2	2	1.5	.....	+ healing in 1 case, no healing in 1 case.
25	3	3	4.0	8.0	+++ healing in 2 cases, ++++ healing in 1 case.
	7	5	6.0	6.2	++++ healing in 6 cases, +++ healing in 1 case.
	11	7	6.5	6.4	++++ healing in all cases.

The longer period of exposure in the curing process of the alfalfa resulted in marked increase in its calcifying properties. Sufficient vitamin D had been synthesized to induce complete calcification of the rachitic bones, in the majority of the rats tested, in a period of five days when the alfalfa leaves fed were at the low level of 5 per cent.

DISCUSSION

Further evidence that the antirachitic potency of alfalfa hay is directly related to the duration of its exposure to sunlight is presented by the data tabulated above. The conditions of the curing

process are therefore of considerable importance, for increasing the calcifying properties of alfalfa enhances its feeding value.

Samples of alfalfa which had been cured for shorter periods than 24 hours were not tested for vitamin D as at first planned for synthesis of vitamin D in the 24-hour curing period was not of such a high degree to make it seem worth while to test alfalfa which had even less opportunity for this synthesis.

In spite of the fact that allowing alfalfa to lie on the field after cutting undoubtedly increases its calcifying properties in proportion to the length of exposure to sunshine, this does not seem a justifiable procedure from the practical standpoint. In a previous paper (*5*) it has been shown that destruction of vitamin A increases very greatly as the time of exposure is prolonged. The alfalfa allowed to lie on the field under the conditions of this experiment showed some increase in its antirachitic potency, but there was an accompanying destruction of practically 84 per cent of the vitamin A content, although bleaching of the green color was not excessive. When synthesis of vitamin D was great, as in the first week of exposure, the alfalfa was severely bleached and comparatively devoid of vitamin A.

The curing of alfalfa in Arizona can perhaps be considered more of a dehydration than a maturing process. It is the usual custom of the farmers in this State to collect the alfalfa in windrows after it has wilted (several hours), and bale it from two to five days after cutting. The alfalfa as marketed, therefore, has in most cases been exposed to the direct sun's rays less than the alfalfa under test which was carefully spread out by hand and allowed to lie on the field from 11.15 a. m. one day until 12 noon the next day.

Increased exposure of alfalfa to sunlight for the purpose of enhancing its calcifying properties apparently is not a procedure to be recommended because of the accompanying marked destruction of vitamin A, as well as possible weather damage, loss of leaves, and other disadvantages.

CONCLUSIONS

The data presented in this paper give additional evidence that alfalfa cured in the dark is deficient in antirachitic potency and that synthesis of vitamin D occurs when the alfalfa is exposed to sunlight in the curing process.

In the experiments described, prolonged feeding of the alfalfa cured in the dark resulted in no alleviation of the induced rachitic condition of the experimental rats.

Alfalfa that had been carefully spread out on the field from 11.15 a. m., one day to 12 noon the next, during which time the sun shone for 15 hours and 5 minutes, possessed mild calcifying powers.

Alfalfa which had been allowed to lie in the swath for one week, during which time the sun shone 57.3 hours and 0.37 inch of rain fell, was found to be highly antirachitic.

The practicability of increasing the vitamin D content of alfalfa and hence its feeding value by prolongations of its exposure to sunlight in Arizona is discussed, but such procedure is not recommended because of serious detriment to the hay.

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INFECTION OF CORN PLANTS BY PHYSODERMA ZEAE-MAYDIS SHAW [1]

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INTRODUCTION

Brown spot has been reported as causing losses estimated at 5 to 10 per cent of the corn crop in certain sections in the South Atlantic and Gulf Coast States;[3] in the vicinity of Gainesville, Fla., the damage is estimated at 5 per cent. This disease, which is caused by the fungus, *Physoderma zeae-maydis* Shaw,[4] attacks the leaves, leaf sheaths, stalks, and occasionally the outer husks of ears of the corn plant. Tisdale [5] has given most of the information available on the nature, distribution, and economic importance of brown spot and the life cycle of the causal organism.

Since brown spot is one of the most important diseases of corn in the South, experiments were begun at Gainesville, Fla., in 1929 to obtain further information on the disease and to establish methods of procedure by which the relative susceptibility of varieties and pure lines of corn may be tested, preliminary to a study of the possibility of controlling this disease by developing resistant inbred lines which could be combined into synthetic varieties. This work has been in progress three years, and information has been obtained on (1) some of the outstanding characteristics of the disease, (2) an effective method of inoculating plants in the field and greenhouse to produce epidemic conditions, (3) longevity of the fungus and time required for the production of sporangia in host tissue, (4) influence of certain factors on the rate of infection, and (5) comparative susceptibility of certain commercial varieties and inbred lines to the disease.

HOSTS

Thirty-eight southern varieties of *Zea mays* var. *indentata* Sturt. (dent corn) and *Z. mays* var. *indurata* Sturt. (flint corn) grown in test plots proved to be susceptible to brown spot, and infection experiments also showed that *Z. mays* var. *saccharata* Sturt. (sweet corn), *Z. mays* var. *everta* Sturt. (pop corn), *Z. mays* var. *amylacea* Sturt. (flour corn), and *Z. mays* var. *tunicata* Sturt. (pod corn) are also susceptible to the disease. The fungus is a common parasite in the leaves of *Euchlaena mexicana* Shrad. (teosinte). Other plants that were artificially inoculated but failed to show brown-spot infection were: *Tripsacum dactyloides* L. (gama grass), and *Coix lachryma-jobi* L. (Jobs-tears) near relatives of the corn plant; and *Holcus sorghum* L. var. *saccharatus* (L.) L. H. Bailey (sorgo), *H. sorghum* L. var.

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[2] Thanks are due F. H. Hull and J. D. Warner, assistant agronomists of the Florida Agricultural Experiment Station, for access to their experimental plots for the purpose of making observations.

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[5] TISDALE, W. H. Op. cit.

technicus (Körn. and Wern.) L. H. Bailey (broomcorn), *Pennisetum typhoideum* Rich. (pearl millet), *Panicum miliaceum* L. (broomcorn millet), *Chaetochloa magna* (Griseb.) Scribn. (Everglade millet), *C. viridis* (L.) Scribn. (green foxtail), *C. lutescens* (Weigel) Stuntz (yellow foxtail) and *Saccharum officinarum* L. (sugarcane).

SYMPTOMS

Since Tisdale [6] has described the manner of infection and symptoms of brown spot, only the outstanding characteristics of the disease are mentioned in this paper. Brown spot is most often located on the plant parts below the ear or even between the fourth node and the ground. In the most severe cases of infection, all or almost all the leaf blades and sheaths on a stalk become diseased and die prematurely, resulting in a weakened plant which bears only a small ear or none. (Fig. 1.) Leaf sheaths near the base of the plant are the most common points of infection, and the culm in this region is sometimes invaded, causing the plant to break, particularly if the disease has girdled it. (Fig. 2.) Leaf-blade infection occurs in four forms: (1) As alternating bands of diseased and healthy tissue (fig. 3, A); (2) as a chlorosis of the tips and in some cases all or a major portion of the entire blade, in which the infected areas are filled with small brown spots containing sporangia (fig. 3, B); (3) as brown areas at the junction of leaf blade and sheath sometimes extending up the midrib of the leaf and down into the sheath, which may become so severely diseased that the water and food supply is cut off, resulting in the wilting and premature death of the leaf (fig. 3, C); and (4) as small brown spots coalesced or scattered in the midrib of the leaf blade. All these forms of leaf infection may appear in one leaf or in different leaves of the same plant. The outer husks of ears are rarely infected, but the husks of rudimentary ears which have failed to emerge from the protecting leaf sheath are frequently diseased.

INOCULATION EXPERIMENTS

Tisdale [6] obtained infection of corn plants by spraying a water suspension of sporangia of *Physoderma zeae-maydis* behind the leaf sheaths and into the buds, under certain environmental conditions. In testing the reaction of varieties and inbred lines of corn to brown spot, it is essential that they be exposed to the parasite under favorable conditions for infection to occur. Accordingly, inoculation experiments were conducted in the greenhouse in the winter of 1929–30 and in the field in 1930 to find a convenient and effective method of inoculating large numbers of plants.

IN THE GREENHOUSE

Corn plants were grown in 6-inch flowerpots in a greenhouse where the mean daily range in temperature was 68.5° to 96.6° F. and the mean daily range in relative humidity was 42.4 to 86.9 per cent. Equal numbers of plants 2 to 3 feet in height, of each of 10 selfed lines, which had been inbred from two to three generations, were inoculated

[6] TISDALE, W. H. Op. cit.

by each of two methods in which sporangia suspended in water were used as the inoculum. The results showed that 59.6 per cent of 248 plants inoculated by injecting the inoculum into the stalk at or near

FIGURE 1.—A Healthy plant bearing a normal-size ear; B, brown-spot-infected plant bearing no ear

the growing point became infected, while 32.2 per cent of a like number of plants became infected when the inoculum was poured into the upper whorl of leaves.

Sponrangia suspended in water served as a better inoculum than dry sporangia. Of 40 plants inoculated with a sporangial suspension 23

developed brown spot, while only 7 of 40 plants developed the disease when dry sporangia were used. Apparently the dry sporangia failed to come in contact with the water in many plants, and thus failed to germinate and invade the host, whereas sporangia suspended in water not only had a more favorable environment for germination but were brought into closer contact with susceptible host tissues by the water of the inoculum moving downward in the crevices between the leaves of the plant.

Other experiments were conducted in which corn kernels and soil were inoculated by the following methods: (1) Dusting sporangia on the kernels; (2) plunging the kernels into a water suspension of sporangia; (3) mixing sporangia with the top 2 inches of soil; (4) dusting sporangia on the surface of the soil; and (5) filling the upper whorl of leaves of plants, 2 to 3 feet in height, with a water suspension of sporangia. Forty plants were used in testing each method of inoculation, and notes were taken on infection seven weeks after planting.

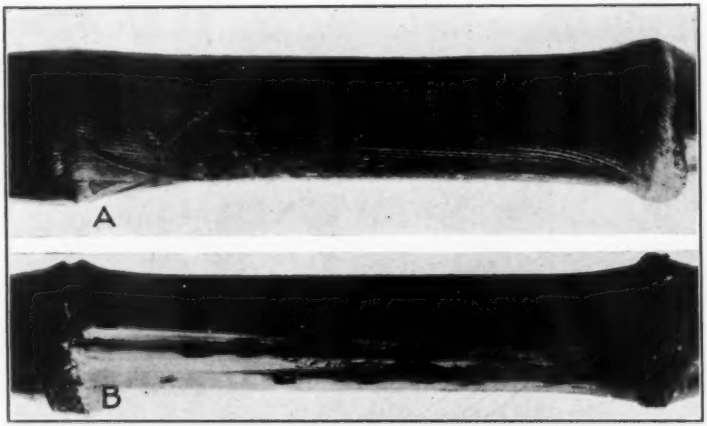

FIGURE 2.—Brown spot infection on sheath (A) and culm (B) of a corn plant

Twenty-three of the 40 plants inoculated by the fifth method developed brown spot, thus showing that the strain of corn used was susceptible to the disease and that the conditions maintained in the greenhouse were favorable for its development. No infection resulted when the other four methods of inoculation were used, although one plant growing in a pot in which the sporangia were dusted on the surface of the soil became infected. It is quite probable that infection in this case resulted either from sporangia that were splashed into the bud or sheath water of the plant when it was watered or from sporangia transported by air currents or insects from the surface of the inoculated soil to the plant.

It is reasonable to assume that under field conditions a much higher rate of infection would result if plants were grown in soil as heavily charged with sporangia as was the soil in which the plants were grown in the greenhouse. Soil in the field is stirred by frequent cultivation, and the top layer at times becomes very dry so that the wind can pick up fine soil particles to which sporangia adhere and deposit them on

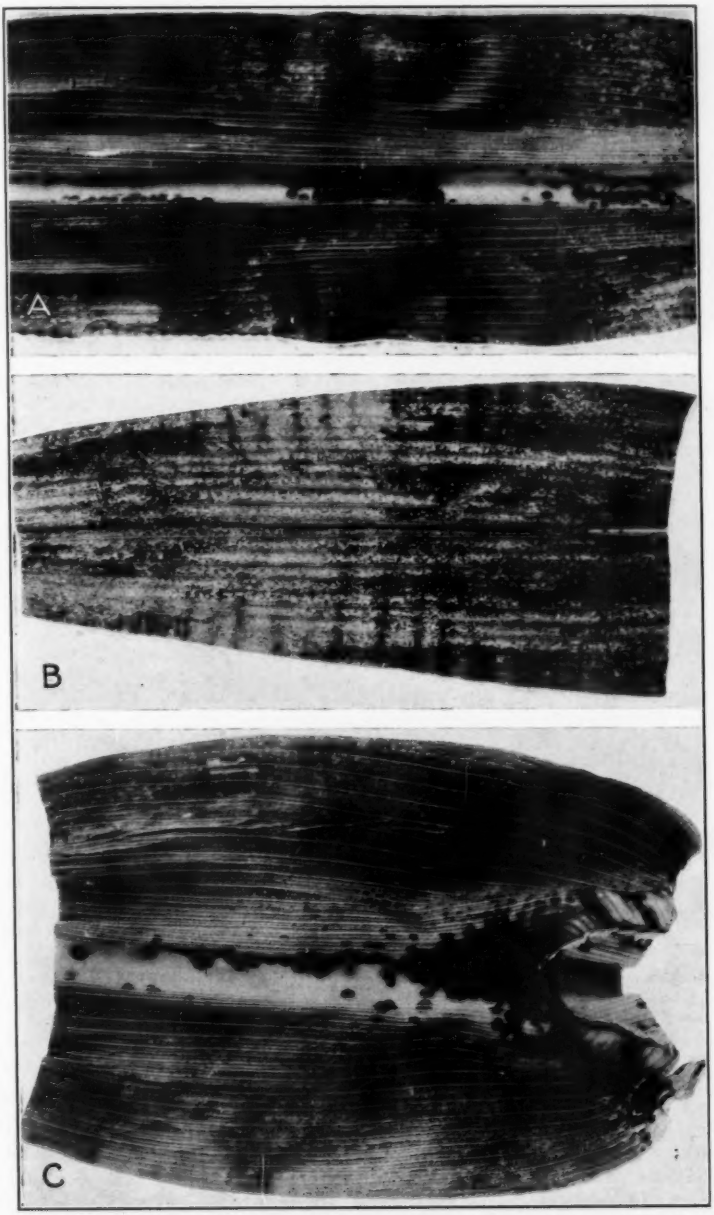

FIGURE 3.—Three forms of brown-spot infection of corn leaves: A, Leaf with alternating bands of diseased and healthy tissues; B, leaf with chlorotic areas near tip; C, leaf with brown areas near junction of blade and sheath

the plant. In the greenhouse the surface of the soil in the pots was not disturbed, and since it was watered daily, probably many of the sporangia were washed downward. The surface remained moist most of the time, and there was little movement of the air above the benches crowded with growing plants.

IN THE FIELD

Corn plants 2 to 3½ feet in height were inoculated with a water suspension of sporangia in the season of 1930 by three methods, as follows: (1) The upper whorl of leaves of each plant was filled to overflowing with inoculum from a quart-size pressure sprayer; (2) inoculum was sprayed on the stalks of the plants from the top to the bottom; and (3) 5 c c of inoculum was injected into the stalks of the plants at or near their growing points with a hypodermic syringe. At the end of 50 days the highest percentage of brown spot appeared in plants which had had the upper whorl of leaves filled with inoculum. (Table 1.)

The injection method gave the highest rate of infection in the greenhouse, and the probable explanation for its being less effective than other methods in the field is that the injections were not made at the right point in the stalks of the plants. In the greenhouse the plants were very uniform in size, and, since they were grown in pots, they could be handled easily, thus enabling the operator to locate fairly readily the growing points and make the injection. In the field the plants varied in size, and, since they could not be moved about, it was difficult to find the growing points and inject the inoculum into them.

TABLE 1.—*Comparative effectiveness of three methods of inoculation in producing brown spot in corn plants in the field in 1930*

Method of inoculation	Plants inoculated	Plants with brown spot at end of 50 days
	Number	*Per cent*
Sporangial suspension sprayed into the upper whorl of leaves of the plants	316	96.5
Sporangial suspension sprayed on the stalks of the plants	324	79.3
Sporangial suspension injected into the plants	327	74.8
Plants not inoculated (checks)	315	12.7

COMPARATIVE EFFECTIVENESS OF GREENHOUSE AND FIELD INOCULATIONS

Comparisons were made of the percentages of brown spot developing in six inbred lines of corn artificially inoculated and grown in the greenhouse in the winter months of 1929–30 and in the field in the summer of 1930 to determine whether the inbred lines reacted the same toward the disease in the two places. The seed of each line planted in both places was from the same ear, and the plants that were in a susceptible stage of growth were inoculated by filling the upper whorl of leaves with a water suspension of sporangia of *Physoderma zeae-maydis*.

In these tests two lines, Wilson 57–39–1–3 and Boys Corn Club 65–1–5, were outstanding for susceptibility in the greenhouse, and,

although the percentage of infection was lower, they were also most susceptible in the field. The other four lines showed a comparatively low percentage of infection in the greenhouse and a much higher percentage in the field, although the order of their resistance was changed. Furthermore, the total percentage of plants infected in the field was almost twice that of those infected in the greenhouse, thus showing that the reaction of inbred lines to brown spot in the greenhouse is not a true indication of their reaction under field conditions. (Table 2.)

TABLE 2.—*Comparison of the percentages of brown spot produced by artificial inoculation in inbred lines of corn grown in the greenhouse and in the field*

Variety and inbred line number	Inoculated in greenhouse			Inoculated in field		
	Total plants	Infected plants		Total plants	Infected plants	
		Number	*Per cent*		*Number*	*Per cent*
Gist 2-3-13	36	6	16.6	77	55	71.4
Mathers 12-2-1	15	5	33.3	22	12	54.5
Cuban Yellow Flint×Florida Flint 18-2-8	47	12	25.5	89	41	46.1
Cuban Yellow Flint×Florida Flint 18-2-1-6	45	5	11.1	78	44	56.4
Wilson 57-39-1-3	31	27	87.1	63	52	82.5
Boys Corn Club 65-1-5	17	16	94.1	12	9	75.0
Total	191	71	37.2	341	213	62.5

LONGEVITY OF SPORANGIA

Tisdale has shown [8] that sporangia of *Physoderma zeae-maydis* remain viable over winter in Alabama, Florida, Mississippi, and South Carolina. A test was started in 1928 to determine how long sporangia would remain capable of causing infection when kept under different environmental conditions. Sporangia were scraped from dead infected corn leaves and leaf sheaths, passed through sieves to remove fragments of host tissue, and then treated as follows: (1) Sporangia were mixed with potting soil, sandy loam soil, and sandy soil, and stored in test tubes which were plugged with cotton, sealed with paraffin, placed in an open flowerpot, and buried in the ground at a depth of 1 foot; (2) sporangia were stored in bottles kept on a shelf in a cabinet in the laboratory; and (3) sporangia in naturally infected leaf sheaths were placed in an open box nailed to the side of a building, exposed to the weather. Table 3 shows that sporangia stored in the three types of soil for three years produced brown spot, and that the sandy soil was a more favorable medium than was either the potting soil or the sandy loam soil. Sporangia kept in bottles in the laboratory for one and two years gave very high percentages of infection, but after three years there was a decrease in their ability to produce the disease. After two years in leaf sheaths the sporangia had lost little, if any, of their power to cause brown spot.

[8] TISDALE, W. H. Op. cit.

TABLE 3.—*Comparative percentages of infection obtained by inoculating corn plants in the field in 1931 with sporangia of Physoderma zeae-maydis of different ages and stored under different conditions*

Year sporangia were collected	Age of sporangia	Conditions of storage	Plants infected [a] by the sporangia
	Years		*Per cent*
1928	3	Potting soil	64
1928	3	Sandy loam soil	49
1928	3	Sandy soil	85
1930	1	Laboratory bottle	99
1929	2	do	94
1928	3	do	41
1929	2	Leaf sheath	87
Not collected (check)	Unknown.	Soil and plant débris	4

[a] Each determination based on 100 inoculated plants of the Tisdale variety of corn.

By inoculating corn plants growing in the field in 1930 with sporangia taken from infected areas in green leaf sheaths, it was demonstrated that they will germinate and cause infection as soon as they are mature. Forty-one plants of the 90 inoculated with such sporangia developed brown spot, while only 6 of the 96 plants not inoculated became diseased.

In the main corn-growing section in north Florida it is believed that most of the brown spot developing during any one season is caused by sporangia produced one or more seasons previously, because sporangia are not liberated from the infected tissues of early-planted corn until the main crop is in an advanced stage of maturity and not susceptible to infection. It is possible that secondary infection takes place to some extent in the southern part of the State, as the planting season is longer there than in northern Florida. Sporangia probably can be carried great distances by the wind.[9] If so, some of the brown spot in corn fields in the northern part of the State may be due to sporangia produced in the southern part during the same year.

TIME REQUIRED FOR THE PRODUCTION OF SPORANGIA IN HOST TISSUE

Data were taken to determine the time required for sporangia to develop in inoculated plants grown in the greenhouse in the winter of 1929–30 and in the field in the summer of 1930. Inoculations were made in the greenhouse by the hypodermic-injection method and by filling the buds of the plants with a water suspension of sporangia. In the field only the latter method was used. When inoculated, plants in the greenhouse ranged in height from 1 to 3 feet and those in the field from 2 to 3½ feet.

As shown in Table 4, 11 days elapsed between the date of inoculation and the first appearance of sporangia in the infected tissues of corn plants growing in the greenhouse. The maximum number of plants with brown spots containing sporangia was observed on the twenty-fourth day. In the field, sporangial formation which could have been due to artificial inoculation was first observed on the sixteenth day after the date of inoculation, and there was a rapid increase in the rate of occurrence of sporangia from the seventeenth

[9] STAKMAN, E. C., HENRY, A. W., CURRAN, G. C., and CHRISTOPHER, W. N. SPORES IN THE UPPER AIR. Jour. Agr. Research 24: 599–606, illus. 1923.

to the twenty-fifth day. On the thirty-sixth day sporangia had been formed in diseased areas in 294 of the 315 inoculated plants, while only 7 of the 315 uninoculated plants were diseased.

In both the field and greenhouse the first sign of brown-spot infection was the development of areas which were a darker green than the normal healthy tissue. (Fig. 4.) Two to three days later the spots turned dark brown, indicating the presence of sporangia.

The variation in the time required for the disease to become established and for the formation of sporangia in the tissues of corn plants after inoculation is probably due to the variation in time

FIGURE 4.—An early stage in the development of brown spot in a corn-leaf sheath. The infected areas are a darker green than the uninfected tissue and the dark-brown spots containing sporangia are beginning to appear

required for the sporangia to become exposed to conditions favorable for germination and subsequent invasion of the host tissues.

TABLE 4.—*Time required for the development of sporangia of Physoderma zeae-maydis in artificially inoculated corn plants growing in a greenhouse and in the field*

Days after inoculation	Number of plants in which sporangia were present in—			Days after inoculation	Number of plants in which sporangia were present in—		
	Greenhouse (165 inoculated)	Field			Greenhouse (165 inoculated)	Field	
		315 inoculated	315 uninoculated			315 inoculated	315 uninoculated
10	0			22	49	177	1
11	3			23	50	209	2
12	5			24	58	228	2
13	6			25	58	256	2
14	9	0	0	26	58	263	2
15	11	1	1	27	58		
16	16	2	1	28	58	269	2
17	24	4	1	29	(a)	278	6
18	28	67	1	32	(a)	287	7
19	34	80	1	34	(a)	290	7
20	35			36	(a)	294	7
21	48	128	1				

[a] No observations made.

RELATION OF STAGE OF GROWTH OF PLANTS TO INFECTION

To determine whether there is a relationship between the age and height of corn plants and their susceptibility to brown spot in the field, plants were inoculated at different stages of growth during the seasons of 1929 and 1930. In 1929, 80 per cent of 217 plants with

their tassels emerging, developed brown spot when their stalks were sprayed with a suspension of sporangia, while only 28 per cent of a like number exposed to natural infection developed the disease. In 1930, five plantings of a commercial variety were made at weekly intervals from April 18 to May 16, and all the plants were inoculated with a water suspension of sporangia of *Physoderma zeae-maydis* on June 9. As noted in Table 5, the highest percentages of infection occurred in plants inoculated when they were 38 to 52 days old and measured 30 to 51 inches in height. Infection in plants 24 and 31 days old and 10 to 12 inches high was the same and was from 30.6 to 36.4 per cent less than that in older and taller plants.

The results of the inoculation experiments show that corn plants in the field are most susceptible to brown-spot infection when 45 to 52 days of age and that infection may occur at any stage of growth, beginning when the plants are 10 inches in height and including plants that have reached the tasseling stage. It is possible that infection may occur when plants are less than 10 inches in height, but it is not likely that it occurs after the plants have tasseled, for their tissues begin to mature and harden at this stage and this would prevent the fungus from entering.

In the greenhouse, plants 5 to 6 weeks old and 2½ to 3 feet in height were more susceptible to brown spot than were those inoculated when they were in an earlier or a later stage of growth. The disease failed to develop in plants inoculated when they were less than a foot high.

TABLE 5.—*Comparative susceptibility of corn plants of different ages to Physoderma zeae-maydis in the field in 1930*[a]

Date of planting	Age of plants	Average height	Inoculated		Not inoculated	
			Total infected		Total infected	
	Days	*Inches*	*Number*	*Per cent*	*Number*	*Per cent*
Apr. 18	52	51	121	97.5	107	33.6
Apr. 25	45	35	74	98.6	56	10.7
May 2	38	30	84	92.8	85	10.5
May 9	31	12	53	62.2	82	0
May 16	24	10	81	62.2	69	1.4

[a] All plants were inoculated June 9, 1930, and final notes on infection were taken July 9, 1930.

RELATION OF WEATHER CONDITIONS TO INFECTION

Ten to ninety per cent or more of the corn plants in fields in the vicinity of Gainesville may become infected with brown spot. In the main crop planted in March and early April farther north the disease usually makes its first appearance the latter part of May or the first of June, and maximum infection is usually reached on or before July 15.

According to Tisdale,[10] conditions most likely to cause an epidemic of brown spot in a cornfield where the plants are making a normal growth are (1) the presence of the sporangia of the causal organism, (2) daily temperatures ranging between 23° and 30° C., and (3) the retention of the sheath and bud water in the plants until the sporangia can germinate and cause infection. Weather conditions favoring the maximum development of the disease at Gainesville usually prevail during May and June.

[10] TISDALE, W. H. Op. Cit.

Comparisons were made of the percentages of brown spot in 10 varieties of corn planted March 10, 1930, and March 6, 1931, and grown in the same field on the agronomy farm in both seasons. Nineteen per cent more of the plants were infected with brown spot in 1930 than in 1931, the infection being 53 and 34 per cent, respectively, for these two years. The temperatures in April and May, 1930, were higher than for the same period in 1931; and there was much more rainfall, a greater number of showers, partly cloudy, and cloudy days during the period from April 1 to June 30, 1930, than there were during this same period in 1931 (Table 6), which verifies Tisdale's conclusion [11] that the disease is more prevalent in warm, wet seasons.

TABLE 6.—*Weather conditions prevailing in April, May, and June, 1930 and 1931, at Gainesville, Fla.*

Condition	April		May		June	
	1930	1931	1930	1931	1930	1931
Clear days.....number	9	15	8	14	5	16
Partly cloudy days.....do	15	10	22	16	14	13
Cloudy days.....do	6	5	1	1	11	1
Days precipitation was 0.01 inch or more.....number	6	7	6	10	20	9
Total precipitation.....inches	2.12	2.83	4.99	2.85	12.03	1.53
Mean maximum temperature.....° F.	80.20	78.37	89.22	85.40	85.86	92.57
Mean minimum temperature.....do	57.70	55.00	63.55	61.08	68.30	67.50
Mean temperature.....do	68.90	66.70	76.38	73.26	77.08	80.03

COMPARATIVE SUSCEPTIBILITY OF VARIETIES OF CORN TO BROWN SPOT

None of the varieties of dent and flint corn tested have been found to be immune to brown spot. The disease has been observed in each of 38 varieties planted March 10, 1930, and March 6, 1931, in test plots at Gainesville, Fla. However, these varieties reacted differently to brown spot; in 10 varieties that were grown in both years, infection in 1930 varied from 33.3 to 78.2 per cent, and from 14 to 46 per cent in 1931, as shown in Table 7. The weather conditions in 1931, as noted previously, were unfavorable for the maximum development of brown spot. The varieties did not rank the same in the order of their susceptibility during both years. For example, in 1930 Mizelle and Tisdale stood at the bottom and top of the list, respectively, whereas in 1931 their rank was reversed.

TABLE 7.—*Comparative susceptibility of 10 commercial varieties of corn to Physoderma zeae-maydis in the field*

Variety	Percentage of brown spot		Variety	Percentage of brown spot	
	1930[a]	1931[b]		1930[a]	1931[b]
Mizelle	78.2	14	Wilson	46.8	38
Florida 191	67	20	Lowmans	44.1	36
Snowflake	63.5	42	Kilgore Red Cob Prolific	38.9	30
Dubose	53.4	40	Cuban Yellow Filnt	36.8	38
Whatley	53.2	36	Tisdale	33.3	46

[a] Percentages based on 77 to 101 plants of each variety.
[b] Percentages based on 50 plants of each variety.

[11] TISDALE, W. H. Op. cit.

COMPARATIVE SUSCEPTIBILITY OF SELF-FERTILIZED LINES OF CORN TO BROWN SPOT

Inbreeding was practiced in a number of different varieties of corn grown during epidemics of brown spot the three seasons, 1929 to 1931, to develop inbred lines for the purpose of selecting strains to be used in a study of the inheritance of the disease and in the creation of resistant synthetic varieties. The disease was induced by filling the upper whorl of leaves of the plants when 3 to 4 feet in height with a sporangial suspension of *Physoderma zeae-maydis*. Although the work has been in progress only three years, the results show that it is possible to obtain resistant and susceptible strains. Several hundred inbred lines have been developed and their reaction to brown spot studied. Of the 10 inbred lines, whose reaction to brown spot is given in Table 8, the first 5 strains were developed by selecting and self-fertilizing plants resistant to the disease, while the last 5 were developed without such careful selection.

TABLE 8.—*Comparative susceptibility of 10 inbred lines of corn to Physoderma zeae-maydis as determined by natural and artificial inoculation in the field*

Variety and inbred line number	Percentage of plants with brown spot [a]			Variety and inbred line number	Percentage of plants with brown spot [a]		
	1929	1930	1931		1929	1930	1931
Petree 8-5-4-1	52.9	23.0	8.6	Cuban Yellow Flint × Florida Flint 18-3-6-4	100.0	100.0	83.9
Early Yellow Dent 14-6-6-19	90.6	41.3	21.0	Cuban Yellow Flint × Florida Flint 18-2-10-17	62.8	82.6	90.0
Cuban Yellow Flint × Florida Flint 18-2-8-18	62.8	45.9	9.0	C. H. × F. B.-U. S. D. A. 73-2-9-29 (Richey)	76.6	85.7	100.0
Wilson 57-36-2-4-10	44.0	37.1	12.5	Laguna 81-1-3-21	52.6	65.6	80.6
Laguna 81-3-2-11	43.7	20.0	4.0				
Mathers 12-2-2-8	69.7	34.4	78.8				

[a] Nineteen to 95 plants of each line were grown each year.

SUMMARY

All subspecies of corn proved to be susceptible to brown spot when artificially inoculated.

Brown spot was found to be most often located on the leaf blades and sheaths of corn plants at or below the fourth node from the ground.

The most effective method of producing brown spot in the greenhouse was to inject a water suspension of sporangia into the stalks of the plants, 2 to 3 feet in height, at or near their growing points. In the field the best method of inoculation was to fill the upper whorl of leaves with inoculum from a hand pressure sprayer when the plants were 45 to 52 days of age and 35 to 51 inches in height.

The reaction of inbred lines to brown spot in the greenhouse was not a true indication of their reaction under field conditions.

Infection was produced by sporangia which had been kept in soil in test tubes buried a foot in the ground for three years, in a bottle in the laboratory for three years, in leaf sheaths exposed to the weather for two years, and by sporangia taken from diseased areas in green leaf sheaths.

Eleven days from the date of inoculation was the minimum time required for sporangia to develop in plants grown in the greenhouse; in the field 16 days were required.

More brown spot developed in the wet season of 1930 than in the dry season of 1931.

Varieties of corn differed in their susceptibility to brown spot and did not rank the same in the order of their susceptibility during the years 1930 and 1931.

Inbred lines of corn have been isolated, which differed in their susceptibility to brown spot when they were exposed to this disease.

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THE DISTRIBUTION OF NITROGEN IN TOBACCO WHEN THE SUPPLIES OF NITROGEN AND OF LIGHT ARE VARIED DURING THE GROWING PERIOD[1]

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INTRODUCTION

With the exception of that done recently, nearly all the work relating to the intermediate products of plant metabolism has had its inception in a desire to isolate products for use in industry, or for therapeutic application. These compounds were for the most part by-products of certain plants and not products that are universally found in all seed plants; and they had no relation to fundamental physiological activities. The general types of compounds were alkaloids, glucosides, and certain dyes. When it became known that there is close interrelationship between ions absorbed from the nutrient medium and the amounts of organic materials synthesized, the quest for an explanation of the intermediate processes began.

The limitations of analytical chemistry are such that little information can be obtained by separating the nitrogen components of a single plant. However, plants grown under different conditions of environment should yield results capable of interpretation. The wider the variation in conditions of growth and development the greater should be the ease of correlation.

Certain root diseases of tobacco have suggested the possibility that reductase activity in the diseased plants may be less than that in normal plants. If any appreciable reduction of nitrates occurs in the root hairs, it may be due to retardation in the normal elaboration of nitrogen rather than to nitrogen deficiency or incapacity of the plant to absorb the ion. In the present experiments an attempt was made to determine the approximate location within the plant at which reduction takes place, and the effects of light and of the relative supply of nitrates on the amounts of different nitrogen fractions in the various plant parts.

METHODS

Havana seed tobacco seedlings were planted singly in glass jars. Each plant was held in place by having its roots inserted through a hole in a cork, and cotton placed about the plant stem. The composition of the medium was as follows:

	Mol.
$CaH(PO_4).2H_2O$	0. 0025
$CaSO_4.2H_2O$	. 0026
$MgSO_4.7H_2O$	. 0057
KCl	. 0037
$NaNO_3$	. 0278

In addition, the medium contained 3 parts per million of boric acid and a trace of ferric potassium tartrate. The media were renewed

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every two days. The nitrogen content of the medium as compared with the content of other essential elements was therefore relatively high. This condition was conducive to a rapid growth of plants, characterized by little fiber and much water, and a consequent tenderness and succulence of plant parts.

The concentration of this medium, except for the nitrogen, is lower than that of most standard nutrient solutions. Preliminary experiments indicated that by renewing the media on alternate days good growth was afforded during the time these plants were growing. The experiments by Stiles (*26*)[2] would suggest that lower total concentrations may be used satisfactorily if the plant is provided with salts at more frequent intervals.

In the greenhouse 206 tobacco seedlings were planted in as many jars on May 4, 1931. The plants were allowed to grow in the greenhouse until June 4. At that time they were removed, and 100 were placed in a light room (where the light was of natural intensity and duration), and 100 were placed in a dark room. Each of these two groups of plants was divided into two groups. Fifty of the plants in the light room and fifty in the dark room continued to receive the same nutrient medium. From the other 50 in each room, sodium nitrate was entirely withheld, but the concentration of the other salts was not altered. During the 11 days of the test the temperature varied from 20° to 21° C.

Both groups of plants kept in the light continued to grow normally, whereas the two groups kept in the dark room showed definite signs of etiolation after two days. At the end of 11 days the plants kept in the light showed no unnatural symptoms, while those kept in the dark were not only markedly etiolated but also had lost a few of their lower leaves. The general appearance and flaccid texture of some of the roots of the plants kept in the dark indicated deterioration. At this time all the plants were harvested, and each plant in the four groups was divided arbitrarily into (1) roots (2) stems (3) midvein of leaves, and (4) part of the leaf left after the removal of midvein. Each of the 16 lots of plant material was sealed in a glass jar, placed in a temperature of −9.5° C. and kept frozen at this temperature until removed for analysis.

EXTRACTION

Nightingale, Robbins, and Schermerhorn (*19*) obtained concordant results in the analyses of frozen plant parts and the corresponding fresh materials. Chibnall (*7*) found that drying plant tissue at 40°–50° C. entailed a loss of protein and an increase in ammonia due to autolysis. Thomas (*27*) dried materials at 70° as the preliminary step in analysis. Tottingham, Schulz, and Lepkovsky (*30*) extracted considerably more soluble nitrogen and soluble protein nitrogen from frozen leaves than from the dried forms, but less from the frozen materials than from the fresh materials.

For analysis the total weight of each lot of material was determined, after which small portions were removed for dry-weight determination. These samples were dried at 105° C. in an electric oven for 36 hours. It would have been preferable to use a lower temperature in partial vacuum. However, the percentages of nitrogen fractions of a

[2] Reference is made by number (italic) to Literature Cited, p. 264.

sample dried at 105° differed only slightly from the percentages of a sample which had been dried at 100°. Portions of each lot were removed for total-nitrogen determination. The remainder of the frozen material was ground through a Nixtamal mill. The solid portions of this ground material were ground again through another mill, and in the case of the more fibrous materials, were ground one or more additional times. With water added, the solute and materials in a colloidal state were expressed through cloth. The residue was then placed in a mortar with water and ground with quartz sand, after which the materials were again placed on the cloth and the extraction repeated. Before the first grinding, no water was added. However, water was liberated from the plants, which rapidly began to thaw. After the first grinding, water was added for grinding in the mill and mortar in amounts depending on the amount of material present. The solute was eventually made up to 2,500–3,000 c c.

The residual pulp was analyzed for total nitrogen. The percentage thus obtained was compared with that obtained by subtracting the total water-soluble nitrogen from the total nitrogen.

The amount of protein that could be dissolved out of the residue (after water extraction had been employed) by heating with a 5 per cent sodium chloride solution for one-half hour at 60° C. was usually very small. This would indicate that globulins had been removed in previous extraction from all ruptured cells, or that this type of protein was present only in minute quantities. Slightly more than 0.01 per cent was extracted by means of salt water from the different plant parts.

TOTAL NITROGEN

Ranker (*23*) found the salicylic acid-thiosulphate method for the determination of total nitrogen unsatisfactory where nitrates are present. The writer found that a loss of nitrogen occurred when this method was employed in these experiments.

The method finally adopted was that in which iron is employed for reduction: The procedure was according to that outlined by Pucher, Leavenworth, and Vickery (*21*). A blank test was made to determine the purity of the iron.

WATER-SOLUBLE PROTEIN

A 200 c c portion of the extract was treated with Stutzer's reagent and allowed to stand overnight and filtered. The material on the filter was digested in the usual manner and the nitrogen determined by the Kjeldahl-Gunning-Arnold method.

AMMONIA AND OTHER VOLATILE BASES

A 100 c c portion of extract was made alkaline with calcium oxide, and distilled with steam. The distillate was taken up by a 0.02 N H_2SO_4 solution, and this in turn was titrated with 0.02 N NaOH. Magnesium oxide was found to serve as well as calcium oxide. The use of MgO caused frothing in the subsequent determination of nitrate nitrogen. In the present instance basic nitrogen was not separated from ammonia. However, a trial indicated that the alkaloid components were small in amount at this stage of growth.

NITRATE NITROGEN

Various methods and modifications have been suggested for determining nitrate nitrogen in the presence of organic matter. Gallagher (*12*) objected to the reduction of nitrates in acid solution when amino acids are present, as the intermediate compound, nitrous acid, acts to liberate nitrogen. He preferred the Valmari-Devardo method. Pyne (*22*) could not duplicate the analyses when he used the Valmari-Devardo method of Gallagher, and obtained better results by reducing the nitrate nitrogen by the use of titanious hydroxide.

Emmert (*10*) used sulphuric acid with the plant materials and drove the resultant distillate over into a flask containing chlorine dioxide. From this point he used for determining nitrate nitrogen in the distillate that part of the method suggested by Harper (*14*) for soil nitrates, namely, phenol disulphonic acid.

Burrell and Phillips (*5*) used the phenol disulphonic acid method by first eliminating the objectionable carbon compounds which would otherwise have interfered with the final color comparisons.

Breckenridge (*3*) obtained serious error by using the zinc-iron method for reduction of nitrates in fertilizers and concluded that the method should not be adopted as an official one.

Other investigators seem to have obtained better results in the reduction of nitrates with aluminum or an alloy of aluminum in alkaline solution. Pozzi-Escot (*20*) used aluminum. Shedd (*24*) obtained favorable results with a modification of Straud's method of using Devardo alloy.

The method finally adopted by the writer was that outlined by Vickery and Pucher (*31*). The extract from which the volatile bases had been expelled was used. This method gave more consistent results in this particular instance than other methods involving reduction in acid or basic solutions.

AMIDES

Vickery and Pucher (*32*) stated that the hydrolysis to ammonia with HCl varied considerably with the HCl concentration. H_2SO_3 is preferred as an acid for hydrolysis.

A portion of the solution from which the soluble protein had been removed by Stutzer's reagent was freed of ammonia by being distilled with steam after sufficient calcium oxide had been added to make it alkaline. To the ammonia-free portion was added sulphuric acid sufficient to make a 5 per cent acid solution. This was hydrolyzed for two hours, a reflux condenser being used in the boiling process.

After being cooled, the hydrolysate was made nearly alkaline with magnesium oxide, and then sufficient sodium hydroxide solution was added to make it distinctly basic. The ammonia was distilled by steam distillation into an acid solution 0.02 N, and titrated with 0.02 N NaOH.

HUMIN

The solution that remained after the amide nitrogen had been removed as ammonia was filtered while hot, and the residue on the filter paper was washed. The nitrogen of this residue was then determined by the method employed for protein nitrogen.

PROTEOSE NITROGEN

An aliquot portion of extract from which water-soluble protein had been removed was saturated with zinc sulphate after having been made slightly acid with sulphuric acid. The precipitate was allowed to stand overnight and filtered. The nitrogen of this precipitate was then determined by the method employed for protein.

ALPHA-AMINO NITROGEN

The humin filtrate was acidified with acetic acid and the amino acid nitrogen determined by the Van Slyke method. In instances where extreme dilution was necessary, this value was very low. In such instances, ammonia was removed from the original extract and an aliquot portion used for determination.

TABLE 1.—*Dry matter and nitrogen in tobacco plants as affected by exposure to light and by fertilization with sodium nitrate*

[All computations made on dry-weight basis]

GROWN FOR 1 MONTH AND 11 DAYS IN LIGHT, $NaNO_3$ SUPPLIED DURING THE ENTIRE PERIOD

Part of plant	Dry matter		Total N	Soluble protein N	Total water soluble N	Ammonia N	Humin N	Protose N	Alpha-amino acid N	Amide N	Nitrate N
	Grams	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Leaves without midvein	60.258	7.260	3.861	1.091	3.810	0.123	0.003	0.076	0.967	0.464	1.090
Midvein of leaf	16.573	4.950	3.838	.691	3.790	.135	(a)	.380	.517	.518	1.482
Stem	24.365	4.419	4.963	1.114	.....	.296	.233	.662	.810	.408	.817
Root	16.863	5.236	3.173	.891	2.872	.230	.194	.171	.406	.297	.243

GROWN FOR 1 MONTH AND 11 DAYS IN LIGHT, $NaNO_3$ SUPPLIED FOR 1 MONTH AND WITHHELD FOR THE LAST 11 DAYS

Leaves without midvein	51.411	6.869	3.661	1.070	3.642	0.227	0.348	0.135	0.951	0.286	0.569
Midvein of leaf	19.142	4.689	3.236	.669	.....	.190	.001	.142	.978	.295	.882
Stem	13.884	4.436	5.158	.745	.....	.391	.355	.387	.949	.774	.733
Root	16.649	6.017	2.382	.586	.....	.237	.079	.229	.787	.114	.194

GROWN FOR 1 MONTH IN LIGHT AND THE LAST 11 DAYS IN DARKNESS, $NaNO_3$ SUPPLIED DURING THE ENTIRE PERIOD

Leaves without midvein	35.784	7.749	4.823	1.191	.....	0.363	0.095	0.130	0.569	0.573	1.586
Midvein of leaf	19.292	6.797	4.137	.377	3.758	.251	.272	.062	.813	.242	1.830
Stem	24.974	5.286	5.645	.775	5.529	.674	.085	.154	1.635	.454	1.311
Root	23.308	6.343	2.791	.691	2.288	.381	.064	.176	.459	.085	.308

GROWN FOR 1 MONTH IN LIGHT AND THE LAST 11 DAYS IN DARKNESS, $NaNO_3$ SUPPLIED FOR 1 MONTH AND WITHHELD FOR THE LAST 11 DAYS

Leaves without midvein	34.310	8.407	5.753	0.911	5.372	0.404	0.007	0.324	1.181	0.280	1.168
Midvein of leaf	13.620	4.766	5.092	1.148	4.701	.532	.227	.308	1.683	.472	1.812
Stem	12.646	3.954	5.177	.801	5.024	.909	.198	.023	1.197	.609	1.103
Root	12.818	5.695	3.242	.843	2.877	.513	.069	.012	.727	.054	.121

(a) Trace.

RESULTS AND DISCUSSION

Since the plants had grown in a medium supplying an abundance of nitrate nitrogen as compared with the amounts of other plant nutrients, it would appear that during the time the plants were in the light more than the normal amount of nitrogen would be taken up (Tottingham and Lowsma (*29*)). This would probably be followed by a large water intake. It will be noted from Table 1 that some of the plants which had been kept in the dark for 11 days before being harvested showed a slightly higher percentage of dry matter of leaves (without midvein) than did those remaining in the light during the entire growing period. This was due in part to the fact that some of the lower leaves had died as a result of the unfavorable environment.

Smith (*25*) found that several varieties of plants thrive economically best when from 10 to 20 parts per million of nitrate nitrogen are present. This amount was relatively small as compared with the amount used in the present trials, in which obviously more nitrate nitrogen than was necessary was present. Haas (*13*), however, found that 500 parts per million of nitrate gave good results for tobacco growth and that sodium nitrate afforded conditions better for intake of Ca^{++} than the nitrate of calcium, magnesium, potassium, or ammonia. However, the literature on this subject does not justify a conclusion at this time.

The presence of some salts containing essential elements affects the intake and elaboration of nitrogen compounds. Thus Burrell (*4*) found that a deficiency of magnesium caused a lowering of the quantity of soluble and insoluble nitrogen in soybean leaves and stems. A deficiency of calcium also caused a lowering of both soluble and insoluble nitrogen, while potassium deficiency caused a lowering of insoluble nitrogen but little difference in soluble nitrogen.

TOTAL NITROGEN

In these experiments under every condition the minimum percentage of total nitrogen was in the roots. To a degree this is in accord with the work of Kraus and Kraybill (*16*), who found that in the stem of the tomato plant there is a descending gradient of total nitrogen. In the light of recent investigations it would be well to consider how nitrogen compounds may be transported in certain plants by means other than those commonly recognized. Maskell and Mason (*17*) found that total nitrogen, protein nitrogen, or amino acid nitrogen may be associated with longitudinal movement, and suggest that the presence of sieve pores in cotton plants should allow the movement not only of crystalloids but also of materials in a colloidal state. The stems of these tobacco plants contained the highest percentage of total nitrogen as compared with that of other plant parts. The total nitrogen percentage of the plants grown in the dark was slightly higher, but the total quantity of nitrogen in the plants grown in the light was slightly greater on account of the increase in growth. The leaf (minus midvein) came next in order in percentage of total nitrogen, with the exception of that of the plants growing in the dark for 11 days without a supply of nitrogen. In that instance the leaf contained more than did any other one part of the plant. These plants showed abnormalities, not only in appearance but in content of nitrogen fractions.

SOLUBLE PROTEIN

At an early stage of development of tobacco the soluble protein compounds seem to be almost equally abundant in the leaves and stem, but less abundant in the roots and veins.

The protein here described probably includes not only albumin but globulins or prolamines, etc., which might be present in minute amounts. Although the last two classes are regarded as not soluble in water, the method of extraction with water by pressing through cloth would not necessarily prevent the insoluble compounds in a fine state of division from being a part of the extract.

AMMONIA NITROGEN AND AMIDES

In all instances the largest percentage of ammonia nitrogen was found in the stem.

In the case of amides no relationship could be shown between the amide content of the plant parts and the different conditions to which the plants were subjected, although the higher percentages were always confined to the aerial portions of the plant. No evidence was found to show that amides may be used for protein synthesis when amino acids are not present in the required amount. The fact that this condition was not arrived at was due to the presence of a constant supply of alpha amino acids during the growing period. It might be mentioned in this connection that amides have been regarded by some workers as parts which form proteins when there is a scarcity of amino acids as in cases of pronounced etiolation (*6*; *15*, *p. 247*).

The conditions referred to, prevailed when amide nitrogen was the product of metabolism. On the other hand, when asparagine was used as a source of nitrogen in a growing medium for tobacco under sterile conditions, the plants reported on by Beaumont, Larsinos, Piekenbrock, and Nelson (*2*) obtained fair growth, though not entirely normal.

Ammonia has been regarded both as a primary and end product in nitrogen metabolism of plants.

ALPHA AMINO ACIDS

Amino acid accumulation in darkness, as indicated by Table 1, is probably due not to more active reductase activity but rather to a piling-up effect incident to the slowing up of processes having to do with the formation of more complex nitrogen compounds. The other explanation would be that tobacco plants, which at this growing stage are sensitive to total absence of light, begin soon to react in the dark to break down proteins and use a nonnitrogenous part of the cleavage product to conserve the carbohydrate supply. The nitrogenous portion might thus eventually appear as alpha amino acids. The latter suggestion seems conjectural in view of the fact that the plant contains relatively little glucoprotein.

NITRATES

Eckerson (*8*) found that in the tomato plant reduction took place at the stem tip just behind the growing region in the leaf cells, especially near the phloem and in the cortical cells of the petioles; in the stem, especially near the nodes, in the phloem paranchyma and the cells in the cortex near the phloem.

In contrast to these annuals, in some plants which have a more or less developed carbohydrate storage system, much of the nitrate nitrogen seems to be reduced at the roots. The work of Eckerson (*9*) and of Thomas (*28*) would indicate that in the apple reductase activity was more pronounced in the roots than in the aerial portion. Also, Nightingale and Robbins (*18*) found that apparently the nitrates are reduced to nitrites and ammonia in the roots of narcissus, with accompanied decrease of carbohydrates in these plant parts.

The outstanding feature of the analytical data is the relatively large percentage of nitrate nitrogen in the aerial portions of the plant in instances where sodium nitrate was supplied during the entire growing period. If the nitrate content of plants grown under favorable economic field conditions is compared with the nitrate content of any of these plants, it will be seen that in none of these was there nitrogen deficiency. This high percentage reaches a maximum of slightly more than 1.8 per cent in the midvein of leaves of plants grown in the dark during the last 11 days before being harvested. The maximum of nitrate nitrogen was found in the mid-rib of the leaf, as compared with the amounts in other parts of leaf, stem, and root. This was true for plants grown in the light and in the dark and regardless of whether the nitrates were withdrawn or continuously supplied. The plants seemed to have taken up a sufficient nitrate supply previous to the 11 days, when nitrogen was withheld from one-half of the number to maintain apparent normal metabolism during this time, providing normal light was retained. It would have been desirable to continue growing the plants in the light until the group no longer receiving nitrogen had nearly, or totally exhausted its accumulated supply of nitrates. If all plants were to be harvested at the same time, however, this could not be done, inasmuch as the plants growing in the dark were intolerant of a more prolonged period in the dark.

Anderson (*1*) found with a variety of plants that the nitrate content varies greatly according to the immediate environment, such as locality of growth, degree of shade, nitrate supply of soil, month of year, and hour of day. These factors finally resolve themselves into the supply of nitrates and the rate of anabolism.

Frear (*11*) found that the small leaves of beet roots contained more nitrate nitrogen than the larger ones, and that the midrib contained always more than the rest of the leaf.

In the experiments with these tobacco plants the leaf portion (other than midrib) decreased in nitrate content to a greater degree than other plant parts as a result of the withdrawal of nitrate from the medium, regardless of light conditions. The next in order of decrease in nitrate content are roots, stems, and midvein. It must be emphasized, however, that there was no evidence of nitrogen starvation, as unelaborated nitrogen was found in all plant parts. The nitrate content of plants grown in the dark showed a marked increase. This would usually be explained as being due to the fact that the gradient of photosynthesis did not keep pace with the rate of respiration, owing to carbohydrate deficiency.

The evidence at hand would seem to support the conclusion that much of the nitrate reduction in tobacco takes place in or near the veins of the leaf.

TOTAL DRY WEIGHT

Under the most favorable conditions of the experiment, when plants were supplied for the entire growing period with nitrates and light, the total dry weight was the highest. There was little difference in the total dry weight of the plants growing during the entire period in the light, from which nitrogen had been withheld during the last 11 days, and that of those to which nitrogen had been supplied during the entire growing period but which had been held in darkness during the last 11 days. The minimum total dry weight was that of the plants from which both light and nitrates had been withheld during the last 11 days. The total dry weights in grams of the groups, in the order in which they have been described, were as follows: 118.059, 101.086, 101.358, and 73.394.

The amount of dry matter in the roots and stems of the plants from which nitrogen had been withheld was materially lower than that in the plants grown under similar conditions of light but receiving a constant supply of nitrogen. This suggests that nitrogen deficiency first affects the root and stem systems. The amount of dry matter of the entire leaf portion of the plants from which light had been withheld was lower than that of plants grown under similar condition of nitrate supply but receiving a constant supply of light. The absence of light first exerted a deleterious effect on the leaf.

SUMMARY

Tobacco plants were grown in the greenhouse in water cultures for one month. At the expiration of this time one-half of the plants were placed in the dark and one-half were continued in the light (in light of natural duration and intensity). Sodium nitrate was continuously supplied to one-half of each of the two groups; from the other one-half of each group nitrate nitrogen was withheld for 11 days before the plants were harvested.

The plants were divided into four plant parts (roots, stems, midvein of leaf, and leaf other than midvein) and analyzed for various nitrogen fractions.

The stems were relatively richer in total nitrogen and ammonia that the other plant parts. The leaves were characterized by a relatively high content of dry matter and protein.

Plants deprived of a nitrogen supply for 11 days still maintained an ample supply of nitrate nitrogen in the tissue.

The plants grown in the dark for 11 days contained a higher percentage of nitrate nitrogen than the corresponding plants grown in the light.

The plants grown in a liberal supply of nitrate nitrogen, with a disproportionately low supply of other plant nutrients, contained a low percentage of dry matter and a high percentage of nitrate nitrogen, indicating a low metabolic rate. These factors did not apparently interfere with luxuriant growth at this stage of the plant's development.

The nitrate and alpha amino acid nitrogen content of the plants seemed to be most influenced by light. Darkness in most instances increased the percentage of nitrogen.

In all instances the maximum percentage of nitrate nitrogen was found in the midvein of the leaf. This would suggest that considerable reduction of nitrogen occurred in or near the veins of the leaf.

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STUDIES ON THE DISSOCIATION OF THE BRUCELLA GROUP[1]

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INTRODUCTION

Since the bovine and porcine strains of Brucella have been demonstrated to have pathogenicity for man, new interest in this group of organisms has resulted in a rather extensive and inclusive study of these organisms. A number of methods have been developed by which these organisms may be classified, according to source, into three groups; namely, caprine, bovine, and porcine types. The methods have not always given the same results in the hands of various workers; hence the question of reliability of these procedures arises.

Unfortunately, perhaps, none of these systems of classification takes into consideration the possible rôle that dissociation might play in inducing variability of the organisms. Hadley (*3*)[2] cites numerous instances of dissociation changes in an organism, causing not only marked colonial appearance but also disturbances in physiological and antigenic properties. The possibility of such changes in members of the Brucella group should be seriously considered—in fact, no system of classification can be considered accurate which fails to recognize the influence of dissociative changes. The significance of dissociation in this group has been only partly studied.

HISTORICAL REVIEW

It seems needless to review the early literature concerning the appearance of atypical or paramelitensis type Brucella cultures. The first description of R types in *Brucella abortus* was presented by Henry (*4*) who demonstrated such forms by spontaneous and enforced dissociation. He describes an opaque, granular colony that gave spontaneous agglutination. In a later publication (*5*) he states that by agglutinin absorption tests the R types of *Br. suis* were distinct serologically from the *Br. abortus*. Marshall and Jared (*10, p. 321, 323*) describe R types of Brucella as follows:

> Older colonies showing definite rough papillæ are so sharply defined as to warrant little description. They are decidedly opaque and granular, and have irregular edges. * * * The R colonies used were usually larger and were distinctly more opaque than S colonies; their structure was more granular, relative to the homogeneous structure of S colonies; in confluent growth of colonies a very sharp mosaic of spindle and other zones of demarcated growth bounded by arcs appeared * * * ; edges might appear irregular, although this was the exception rather than the rule; crystals, not uncommon on stock colonies, were perhaps more frequently encountered on R colonies. Growth of R colonies was more rapid, a fair growth occurring in 24 hours. The outstanding features were increased opacity and granular central structure; in exceptional cases one might definitely

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[2] Reference is made by number (italic) to Literature Cited, p. 279.

see these points macroscopically. * * * Cellular morphology was not observed in great detail; the R type cells, however, appeared longer.

In a rather extended study of dissociation, Zdrodowski, Brenn, and Voskressenski (*13, p. 793*) describe R types as follows:

Les colonies rondes, mais moins bonbées et parfois presque plates, avec des contours irreguliers; leur structure est grossière, granuleuse; la pigmentation est verdâtre. Les cultures sur gélose s'émulsionment mal dans la solution de NaCl; leur émulsion n'est pas stable (agglutination spontaneé) et fournit en genéral une thermo-precipitation positive d'après Burnet. Les cultures en bouillon sont en floccons et peu a peu s'eclaircissent en format un sédiment. Le pouvoir agglutinogène est nul ou trés faible. Les cultures sont avirulentes ou très faiblement virulentes pour les cobayes.

Soule (*12*) claims to have obtained typical R types as well as mucoid forms.

HISTORY OF CULTURES

The cultures used in these studies were obtained from I. F. Huddleson from the central Brucella station. The history of the cultures follows:

Brucella abortus 1.—This culture was obtained from the Bureau of Animal Industry, United Stated Department of Agriculture, prior to 1915. The source and date of isolation are unknown.

Br. abortus 2 and 3.—These organisms were isolated from aborted fetuses in 1915 from herd A at Michigan State College.

Br. abortus 4.—This strain was isolated from the udder of a cow from experimental herd of Michigan State College in 1915.

Br. abortus 5.—This culture was in the laboratory stock cultures prior to 1915. The source and date of isolation are not known.

Br. suis 400.—This culture was obtained from Dr. Don M. Griswold, of the Michigan Department of Health. It was isolated from a boar's testicle. The date of isolation is not known.

Br. suis 401 and 402.—These cultures were obtained from Purdue University. Culture 401 was isolated in 1925. The source and date of isolation of culture 402 are unknown.

Br. suis 404 and 405.—These cultures were obtained from J. W. Connaway, of the University of Missouri, in 1922. Culture 404 was isolated from a premature fetal pig from a naturally infected sow in 1922. Strain 405 was isolated from swine. The date of isolation is unknown.

Br. suis 408.—This strain was obtained from Professor Good, of the University of Kentucky. It was isolated from a hog. The date of isolation is unknown.

Br. melitensis 301.—This organism was isolated by J. P. Torrey. The date of isolation is not known.

Br. melitensis 312, 315, and 316.—These cultures were obtained from Doctor Burnet, of the Pasteur Institute, Tunis, Algeria. They were isolated from cases of undulant fever. Culture 316 was isolated in 1929.

Br. melitensis No. 318.—This organism was received from K. F. Meyer, of the William Hooper Foundation of the University of California. The source and date of isolation are not known.

EXPERIMENTAL PROCEDURE

BACTERIOLOGICAL STUDY OF THE CULTURES

Prior to the dissociation studies presented in this paper, all the cultures used were plated repeatedly to eliminate all possible contamination and to reduce the possibility of mixed strains in the same culture. The pure-line strains thus obtained were then studied culturally, physiologically, and serologically to check their identity as to type. Carbohydrate fermentation tests were made on glucose, mannitol, maltose, sucrose, and lactose. Serological tests were made by test-tube agglutination method using a positive *Brucella abortus*

serum. Smears were made on liver infusion agar to determine colony appearance. For comparative purposes, it was deemed advisable that all strains should be typical smooth forms. All cultures were classified as to species by means of dye sensitivity (Huddleson (*7*)) and hydrogen sulphide production (Huddleson and Abell (*8*)). All the strains were typed as named, and no atypical results were obtained that would indicate the presence of any contamination or mixed strains of Brucella.

The extreme care exercised in identifying and purifying the cultures selected for study is indicated here to show that the cultures were all pure-line strains of Brucella. This preliminary study was made to eliminate contamination as effectively as possible, particularly as the data presented later in this paper revealed the fact that the R types of Brucella were culturally, antigenically, physiologically, and morphologically different from their S prototypes.

METHODS USED TO INDUCE MICROBIC DISSOCIATION

EXPERIMENT 1

Growing S strains in the presence of 10 per cent S antiserum has caused dissociation in such groups as the paratyphoid, pneumococci, and spore-forming bacilli, as well as many others. Accordingly, this method of producing R types was tried. All the cultures of the Brucella group were seeded into 10 per cent S antiserum broth, transferred serially, and plated at intervals of 48 hours. A second series of these cultures was aged in 10 per cent S antiserum broth and plated at weekly intervals. Colonies that showed rough colonial appearance were planted on liver agar slants and studied for stability of type before they were classified as rough forms.

TABLE 1.—*Dissociation changes by members of the Brucella group induced by growing in 10 per cent S antiserum broth*

[Plated at intervals of 48 hours]

Cultures	Type of colony * at indicated time of transferring																
	April 30	May 2	May 4	May 6	May 8	May 10	May 12	May 14	May 16	May 18	May 20	May 22	May 24	May 26	May 28	May 30	June 2	June 4
Br. abortus:																		
1	–	–	S	S	S	S	S	–	S	S	OS	–	OS	OS	OS	OS	OS	–
2	S	S	S	S	S	S	S	S	S	S	OS	–	–	–	OS	OS	OS	OS
3	S	S	S	S	S	S	S	S	–	–	OS	OS	OS	OS	OS	OS	OS	OS
4	–	S	S	S	–	S	S	S	S	–	OS	OS	OS	OS	OS	OS	OS	OS
5	–	–	–	S	S	S	S	S	S	S	OS	OS	OS	OS	–	OS	–	–
Br. suis:																		
400	–	S	S	S	S	S	S	S	S	S	OS	OS	OS	OS	OS	OS	–	OS
401	S	S	S	S	S	S	S	S	S	S	OS	OS	OS	OS	–	OS	–	OS
402	S	S	S	S	S	S	S	S	S	S	OS	OS	OS	OS	–	–	OS	OS
404	S	S	S	S	S	S	S	S	S	S	OS	OS	OS	OS	OS	–	OS	OS
405	S	S	–	–	S	S	S	S	S	S	OS	OS	–	OS	OS	OS	OS	OS
408	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R
Br. melitensis:																		
301	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R
315	S	S	–	S	S	S	S	S	S	S	S	OS	OS	OS	S	S	OS	OS
316	–	–	S	S	S	S	S	S	S	S	S	OS	OS	OS	S	S	OS	OS
312	S	S	S	S	S	S	S	S	S	S	–	OS	OS	OS	–	–	OS	OS
318	–	S	S	S	S	S	S	S	S	S	–	–	OS	OS	R	R	R	R

* S=smooth type; OS=smooth-opaque type; R=rough type; –=no growth.

In the series transferred every 48 hours (Table 1) no rough forms of *Brucella abortus* were found after 18 serial transfers. An opaque granular type colony which Marshall and Jared call R forms, but which the authors designate as opaque smooths (OS), was observed in the eleventh transfer, in all five strains studied. These forms persisted throughout the experiment, with a complete disappearance of the S colonies. In the case of *Brucella suis*, only one strain (No. 408) produced an R form. This appeared after the fifth transfer. The other five strains of *Brucella suis* produced OS forms after the eleventh transfer. Two of the *Brucella melitensis* strains (Nos. 301 and 318) produced R forms after the twelfth and fifteenth transfers, respectively. The remaining three strains of *Brucella melitensis* produced OS forms after the eleventh transfer. In the series that was aged and plated at weekly intervals, similar results were obtained.

Experiment 2

Hadley (*3*) has been quite successful in inducing R forms in members of the paratyphoid group through the agency of lithium chloride. This method was followed in this experiment. Tubes containing 1 per cent solution of lithium chloride in veal broth were seeded with the various strains of Brucella used in the previous experiment. These cultures were transferred weekly, at which time they were plated on liver infusion agar. The experiment was carried through a period of 10 weeks. The data are presented in Table 2. The results are similar to those obtained by the use of 10 per cent S antiserum broth, except that *Brucella melitensis* 301 failed to produce R forms. All strains readily produced the OS type. In general, the use of 1 per cent lithium chloride was not so satisfactory a dissociation incitant as was the 10 per cent S antiserum broth.

Table 2.—*Dissociation changes by members of the Brucella group induced by growing in 1 per cent lithium chloride broth*

Cultures	Type of colony [a] at indicated time of transferring									
	May 6	May 13	May 20	May 28	June 6	June 11	June 18	June 25	July 2	July 9
Br. abortus:										
1	.....	.....	S	OS	OS	OS	OS	OS	OS	.....
2	S	.....	S	OS	OS	OS	OS	.....	OS	OS
3	S	S	S	OS	OS	.....	OS	.....	OS	OS
4	S	S	S	OS	.....	.....	.....	OS	OS	OS
5	S	S	S	OS	.....	.....	.....	OS	OS	OS
Br. suis:										
400	S	S	.....	S	.....	OS	OS	.....	OS	OS
401	OS	S	S	S	.....	OS	OS	.....	OS	OS
402	S	S	S	S	OS	OS	OS	OS	.....	OS
404	S	S	S	S	OS	.....	OS	.....	OS	.....
405	S	S	S	S	OS	.....	OS	OS	OS	OS
408	S	S	S	S	R	R	.....	R	R	R
Br. melitensis:										
301	.....	S	S	S	OS	OS	.....	.....	OS	OS
315	S	S	S	S	OS	OS	.....	.....	OS	OS
316	S	.....	S	S	OS	OS	OS	.....	OS	OS
312	S	.....	.....	.....	OS	OS	OS	OS	OS	OS
318	S	.....	S	S	OS	SR	.....	R	R	R

[a] S=smooth type; OS=smooth-opaque type; SR=smooth and rough type; R=rough type.

Experiment 3

It has been the experience of many laboratory workers with Brucella that frequently old agar slant cultures give rise to abnormal growth. For this reason, agar slant cultures were incubated at room temperature for a period of six weeks and were then transferred to veal infusion broth. With the hope of encouraging any rough tendencies in the strains induced by aging on agar for six weeks, the veal-broth cultures were aged for a period of eight weeks. At intervals during this latter aging period, these cultures were smeared on liver infusion agar to detect any changes that might have occurred. The data are presented in Table 3. At the end of 6 weeks, 3 cultures of *Brucella abortus* (Nos. 1, 4, and 5), and 5 of the 6 strains of *Brucella suis* were rough. Only strain 401 remained S type throughout the experiment. Three of the five strains of *Brucella melitensis* became rough, while strains 312 and 315 remained S type.

Table 3.—*Dissociation changes by members of Brucella group induced by agar or liver agar slant then transferred to plain broth*

[Slant cultures were incubated at room temperature for six weeks]

Cultures	Type of colony * at indicated time of transferring							
	Oct. 5	Nov. 2	Nov. 9	Nov. 16	Nov. 23	Nov. 30	Dec. 6	Dec. 13
Br. abortus (bovine strain):								
1	S	S	S	S	S	R	R	R
2	S	S		S	S	S	S	S
3		S	S	S	S	S	S	S
4	S			S	S		R	R
5	S	S	R	R		S	R	R
Br. suis:								
401	S	S		S	S	S	S	S
400	S	S	S		S	R	R	R
402	S	S	S	S		R	R	R
404	S	R	R	R		R	R	R
405	S	S	S	S	S	S	R	R
408	S	R	R	R		R	R	R
Br. melitensis (caprine strain):								
312	S	S			S	S	S	S
301	S	S	S	S	S	R	R	R
316	S	R	R	R			R	R
315		S	S	S	S	S	S	
318	S	S	S	S	S	R	R	R

* S=smooth type; R=rough type.

The results obtained by this process of dissociation were quite successful, as measured by the large proportion of the cultures that became rough and the slight amount of dissociation induced by the other incitants used.

The Brucella group, as a whole, seems to be very difficult to dissociate, as evidenced by the data obtained, and also by further experiences of the senior writer. The difficulty of dissociating members of the Brucella group is quite in contrast with the ease of dissociating those of the colon-typhoid group.

Certain cultures were refractory to dissociation incitants. In fact, 2 strains of *Brucella abortus*, 1 strain of *Brucella suis*, and 2 strains of *Brucella melitensis* never gave evidence of rough colonial characteristics, unless the ability to produce the OS colony is considered as a rough type. The writers are inclined to minimize the significance of this colony as a stable rough type, because all of these

forms that were studied were decidedly transitory as was indicated by their rapid return to S types by repeated transferring on liver infusion agar and by the preponderance of the S character in colonies. The writers believe that such forms may be obtained that would be quite stable. The organism used as a vaccine by Huddleson (*6*) may be such an organism. It has been the belief of the senior author that these forms are not rough, as measured by colonial appearance, but are a smooth type that has lost, to a large extent, the antigenic properties of an S form with a complete loss of pathogenicity. The unsuccessful attempts to dissociate these strains seem to confirm the statement of the senior writer (Mallmann (*9*)) that certain S strains of the paratyphoid group were unable to dissociate into rough types.

Further work on the Brucella group, since the completion of these studies, indicates that most of the Brucella strains studied were refractory to dissociation.

STUDIES OF R STRAINS

COLONIAL APPEARANCE OF R CULTURES

The colonial appearance of the R cultures of Brucella is very similar in type to the R forms of the Salmonella group. The colonies of the extreme or true R forms are characterized by a very irregular contour, the edges being extremely jagged, resembling the usual soil-spore-former colony. The surface of the colonies is occasionally wrinkled, flat, and spreading, with a dull appearance. Some of the colonies have a brownish tinge, which becomes more marked with age. The rough colonies are shown in Plate 1.

MORPHOLOGY AND STAINING REACTION OF R CULTURES

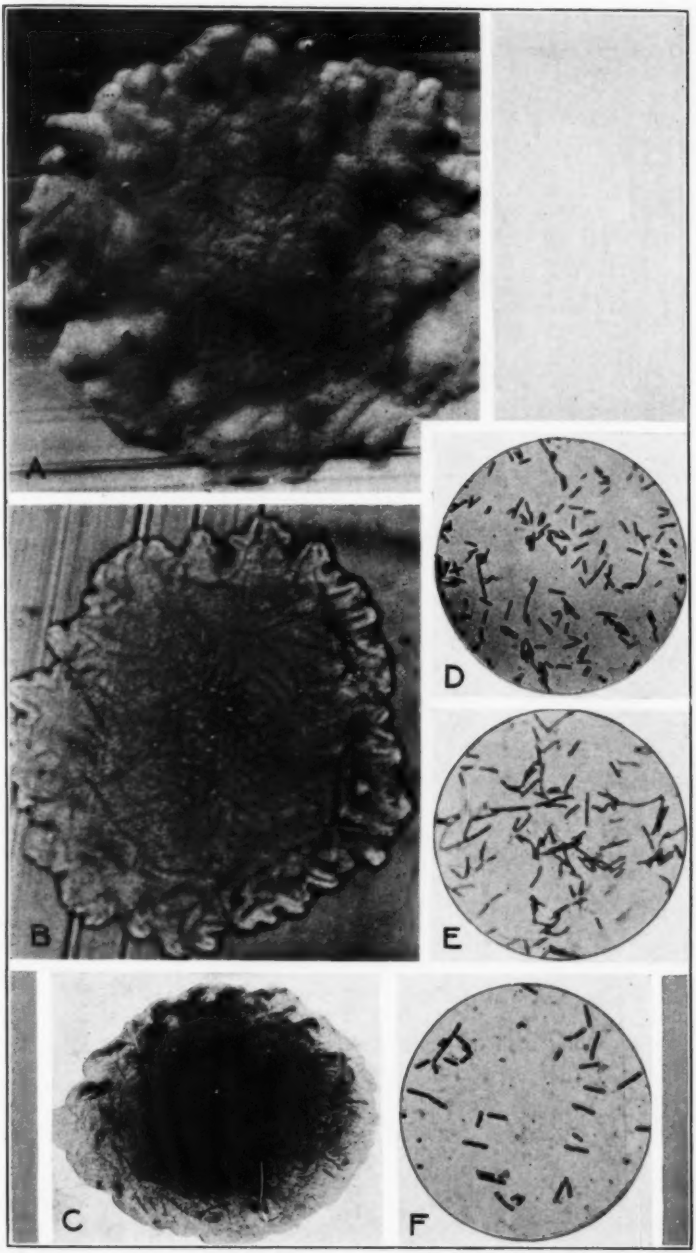
Morphologically, all the R strains of Brucella differed markedly from the S strains, although there was no constancy of shape and size among the R forms. The R forms were, without exception, larger than the S forms, sometimes from three to five times larger. In general, the R forms were long rods, very granular in structure, markedly pleomorphic, with a tendency to produce chains. Marshall and Jared (*10*) state that the R forms they studied were somewhat larger than the S forms. Photomicrographs of the R forms are also shown in Plate 1. In all instances, staining reactions with Gram's stain were similar to those of the original S forms. In fact, Gram's reaction was used as a means of identification, to the extent that all colonies giving posit ve Gram's stain were discarded as being probably contaminated.

BIOCHEMICAL STUDIES OF R STRAINS

Shortly after isolation, all R strains selected for study were repeatedly plated on liver-infusion agar to determine their stability and to insure the elimination of any contamination by foreign organisms and S strains of the organism that might have been carried over in fishing the colony. The strains thus treated were then examined for their behavior on dye plates, production of hydrogen sulphide, fermentative ability, and antigenic properties.

Behavior on Dye Plates

Dye plates containing thionin and basic fuchsin, respectively, were prepared according to the directions of Huddleson (*7*). The plates were seeded with heavy suspensions of 48 to 72 hour liver-



Rough colonies of Brucella: A, *Br. suis*, strain 402R (× 30); B, *Br. abortus*, strain 4R (× 30); C, *Br. melitensis*, strain 301R (× 60). Photomicrographs of B strains of Brucella: D, *Br. suis*, strain 404R (× 736); E, *Br. abortus*, strain 4R (× 736); F, *Br. melitensis*, strain 316R (× 970)

infusion agar slant growths. The suspensions were obtained by washing the growth from agar slant cultures with small amounts of sterile broth. The seeded plates were then incubated at 37° C. for 48 hours, when the results were recorded. All strains grew equally well in both sets of plates. It will be recalled that the smooth forms of *Brucella abortus* are inhibited from growing by the action of thionin, while *Br. suis* is inhibited by the basic fuchsin. The R forms of all three species apparently had a marked tolerance for the dyes. Similar results were obtained by Marshall and Jared (*10*) for their opaque granular types. These experiments were repeated several times, using S forms of each species as a check, with identical results. The results indicate that the R forms could not be classified in a manner similar to that used for their S prototypes.

Production of Hydrogen Sulphide

The hydrogen sulphide production was measured by the method recommended by Huddleson and Abell (*8*). Strips of lead acetate paper were placed inside the tubes and held in position by the cotton stoppers, the paper extending slightly below the stopper. The agar slants were heavily seeded and incubated at 37° C. The strips of paper were removed from the tubes after 24 hours and replaced with fresh ones. This procedure was repeated for seven days. The R strains of *Brucella abortus* did not produce hydrogen sulphide as did their S prototypes. The R strains of *Br. suis* produced less hydrogen sulphide than did the corresponding S strains.

The R strains of *Br. melitensis* behaved the same as the homologous S strains. The rough strains of the Brucella group, as shown by the hydrogen sulphide test, did not behave as do the S strains when typed.

Serological Studies of R Forms

A serological study of the R forms was made to determine the relationship of the various R types and to determine their antigenic relationship to their homologous S types.

Rabbits were immunized by the use of killed cultures of both R and S strains of each species. Each animal received a series of five injections given at weekly intervals. Antigens of all rough and smooth strains were made, the organism being suspended in an 0.5 per cent phenolized salt solution. Cross-agglutinations studies were made on all these antigens using the different antiserums. The data are presented in Tables 4 and 5. The results show that *Brucella melitensis* and *Br. suis* R antigens were agglutinated equally well by both *Br. melitensis* and *Br. suis* R antiserums, and that *Br. abortus* R antigen was not agglutinated by either of these two R antiserums. As would be expected from the above results, *Br. abortus* R antiserum failed to agglutinate R antigens of *Br. melitensis* and *Br. suis*. When these R antigens were tested with S antiserums, prepared by immunizing rabbits with the S prototypes of these R types, no agglutination was induced, although the S forms were all agglutinated equally well. These data, together with the data on morphology, colonial appearance, and physiological properties, emphasize the fact that the R strains dealt with in this paper are R forms of a type similar to those encountered in the typhoid-paratyphoid group. They do not correspond entirely to those described by Henry and by Marshall and Jared.

TABLE 4.—*Agglutinability of R strains of the Brucella organisms using R antiserums*

	Reaction at indicated serum dilutions of antigens										
	C	1–10	1–20	1–40	1–80	1–160	1–320	1–640	1–1280	1–2560	1–5120
Br. melitensis 316R antiserum:											
Br. melitensis 316R	–	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Br. suis 404R	–	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Br. abortus 5R	–	–	–	–	–	–	–	–	–	–	–
Br. suis 400R antiserum:											
Br. melitensis 316R	–	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Br. suis 400R	–	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Br. abortus 5R	–	–	–	–	–	–	–	–	–	–	–
Br. abortus 4R antiserum:											
Br. melitensis 316R	–	–	–	–	–	–	–	–	–	–	–
Br. suis 400R	–	–	–	–	–	–	–	–	–	–	–
Br. abortus 5R	–	++++	++++	++++	++++	++++	++++	+++	+++	+++	++

TABLE 5.—*Agglutinability of R and S strains of the Brucella group using normal smooth serums of the respective species*

	Reaction at indicated serum dilutions of antigens										
	C	1–20	1–40	1–80	1–160	1–320	1–640	1–1280	1–2560	1–5120	1–10240
Br. abortus 2 smooth serum:											
Br. abortus 2	–	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++
Br. melitensis 316	–	++++	++++	++++	++++	++++	++++	++++	++++	++++	++
Br. suis 400	–	++++	++++	++++	++++	++++	++++	++++	++++	++++	++
Br. abortus 5R	–	–	–	–	–	–	–	–	–	–	–
Br. melitensis 316R	–	–	–	–	–	–	–	–	–	–	–
Br. suis 400R	–	–	–	–	–	–	–	–	–	–	–
Br. melitensis 316 smooth serum:											
Br. abortus 2	–	++++	++++	++++	++++	++++	+++	+++	+++	++	++
Br. melitensis 316	–	+++	++++	++++	++++	++++	++++	++++	++++	+++	++
Br. suis 400	–	+++	++++	++++	++++	++++	+++	+++	+++	++	++
Br. abortus 5R	–	–	–	–	–	–	–	–	–	–	–
Br. melitensis 316R	–	–	–	–	–	–	–	–	–	–	–
Br. suis 400R	–	–	–	–	–	–	–	–	–	–	–
Br. suis 400 smooth serum:											
Br. abortus 5	–	++++	++++	++++	++++	++++	++++	+++	+++	++	++
Br. melitensis 316	–	++++	++++	++++	++++	++++	++++	+++	+++	++	++
Br. suis 400	–	++++	++++	++++	++++	++++	++++	+++	+++	+++	+++
Br. abortus 5R	–	–	–	–	–	–	–	–	–	–	–
Br. melitensis 316R	–	–	–	–	–	–	–	–	–	–	–
Br. suis 400R	–	–	–	–	–	–	–	–	–	–	–

No conclusions can be drawn from the behavior of the R strains as to their serological relations, because of the limited number of strains studied. The antigenic relationship of the R *Brucella melitensis* and R *Brucella suis* and the lack of relationship of the R *Brucella abortus* is interesting.

REVERSION OF R FORMS

Because of the totally different characters of the R organisms studied, on the basis of their antigenic, morphological, and physiological properties, it was difficult to establish their identity as R Brucella until they were returned to S forms with their original characters. The methods of reversion used are presented together with the characters of the S organisms obtained.

REVERSION BY RAPID TRANSFERRING AND AGING

Soule (*11*) has demonstrated that an R type, when grown in the presence of 10 per cent R antiserum in broth, will revert to an S type. Mallmann (*9*) has demonstrated that the ability of an organism to revert under these conditions is dependent upon the stage or degree of roughness attained. If the organism carries a residual S character, the use of 10 per cent R antiserum in broth will induce reversion. It has been the experience of the senior writer that R types obtained by laboratory manipulation from S types can generally be reverted with little difficulty. The use of R antiserum was tried by two procedures. In the first, the culture was transferred every 48 hours in 10 per cent R antiserum broth and plated on plain liver infusion agar, and in the second, the cultures were aged in 10 per cent R antiserum broth. The results are presented in Tables 6 and 7. Strains No. 5R of *Brucella abortus* and Nos. 408R and 405R of *Brucella suis* reverted to S types in broth under the influence of rapid transferring and aging. Strain No. 405R of *Brucella suis* reverted under the influence of rapid transferring, but not by aging. Neither of the two strains of *Brucella melitensis* reverted. There was no relation between the ability of an S strain to dissociate to an R type and its ability to revert.

TABLE 6.—*Reversion of R types of Brucella induced by rapid transferring in 10 per cent R antiserum broth*

Cultures	Type of colony[a] at indicated time of transferring											
	Feb. 23	Feb. 25	Feb. 27	Mar. 1	Mar. 3	Mar. 5	Mar. 7	Mar. 9	Mar. 11	Mar. 13	Mar. 15	Mar. 17
Br. abortus:												
4R	R	R	R	R	R	—	R	R	—	R	R	R
5R	R	R	R	—	R	OS	OS	S	S	S	S	S
Br. suis:												
400R	R	R	R	R	RS	RS	—	R	R	R	—	—
402R	R	R	R	R	R	R	RS	R	R	R	R	R
408R	R	R	R	R	R	OS	OS	S	S	S	S	S
404R T	R	R	R	R	R	R	—	R	R	—	R	R
405R	R	R	R	R	R	R	—	R	R	R	R	S
Br. melitensis:												
301R	R	R	R	R	R	R	—	—	R	R	R	R
306R	R	R	R	R	R	R	R	R	—	—	R	R

[a] R=rough type; S=smooth type; OS=smooth-opaque type.

TABLE 7.—*Reversion of R types of Brucella induced by aging in 10 per cent R antiserum broth*

Cultures	Type of colony[a] at indicated time of transferring					
	Mar. 2	Mar. 9	Mar. 16	Mar. 23	Mar. 29	Apr. 5
Br. abortus:						
4R	R	R	R	R	R	R
5R	R	OS	S	S	S	S
Br. suis:						
400R	R		R	R	R	R
402R	R	R	R	R	R	R
408R	R	OS	S	S	R	S
404R	R	R	R	R	R	R
405R	R	R	R	R	R	R
Br. melitensis:						
301R	R	R	R	R	R	R
316	R	R	R	R	R	R

[a] R=rough type; S=smooth type; OS=smooth-opaque type.

During the course of these experiments duplicate platings were made from the 10 per cent R antiserum broth, using plain liver-infusion agar and 1–50,000 gentian violet liver-infusion agar. There appeared to be a marked tendency for the production of S forms on the gentian violet medium. However, the forms thus induced remained S type only when kept on this medium. Transfer to plain liver-infusion agar caused a reversion to the R type, demonstrating that the S forms thus obtained were pseudosmooth colonies. For this reason the results presented in the tables were taken from data secured on the plain liver-infusion agar.

REVERSION BY ANIMAL PASSAGE

Numerous reports in the literature show that animal inoculation of R forms frequently causes reversion when other methods fail. Griffith (*2*) was able to revert R forms of pneumococci to their homologous S forms by injecting the R organism, together with the killed S organisms, subcutaneously into white mice. This was confirmed by Dawson (*1*).

Two methods of reversion were attempted—the inoculation subcutaneously of living R cultures with the killed S prototype and the inoculation of living R cultures alone. The suspension of living cultures was prepared in the usual manner. The killed cultures were prepared by suspending the organism in 0.5 per cent phenolized 0.85 salt solution and subjected to a temperature of 56° C. for one hour. The suspensions thus prepared were plated to check sterility. Equal amounts of living and killed organisms were mixed and diluted to a turbidity of 7 mm on the Gage nephelometer. Living R suspensions were prepared in a similar manner. Guinea pigs were inoculated subcutaneously with the mixtures of killed S and living R organisms and with the living R organisms alone. A series of four injections was given each animal at weekly intervals. The guinea pigs were killed and examined six weeks after receiving the last injection.

TABLE 8.—*Reversion of R types of Brucella by animal inoculation*

Guinea pig No.	Material injected	Pathological changes	Isolation of culture from	Organism isolated
1	Live *Br. melitensis* 316R. Dead *Br. melitensis* 316S.	Liver, enlarged, many grayish-white, foci. Spleen, numerous white foci. Testicles, normal. Lung, greenish-black foci. Kidney, normal. Point of injection, large abscess.	Liver, spleen, lung, abscess.	*Br. melitensis* (smooth).
2	Live *Br. suis* 400R. Dead *Br. suis* 400R.	Liver, normal. Spleen, white foci. Testicles, normal. Lung, greenish-black foci. Kidney, normal. Point of injection, abscess.	Spleen, lung, abscess.	*Br. suis* (smooth).
3	Live *Br. abortus* 4R. Dead *Br. abortus* 4S.	Liver, many pearly white foci. Spleen, enlarged. Kidney, normal. Lungs, normal. Left side of humerous filled with fibrinous exudate.	Liver, spleen, left axillary region.	*Br. abortus* (smooth).
4	Live *Br. melitensis* 316R.	Liver, few white foci. Spleen, normal. Lung, normal. Testicles, normal. Point of injection, small abscess. Inguinal and cerical lymph nodes, swollen.	Liver, abscess.	*Br. melitensis* (smooth).
5	Live *Br. suis* 400R.	Liver, normal. Spleen, normal. Lung, abscess at left diaphragmatic lobe. Kidney, normal. Point of injection, abscess.	Lung, abscess.	*Br. suis* (smooth).
6	Live *Br. abortus* 4R.	Liver, grayish-white foci. Spleen, enlarged, pearly white foci. Lungs, few black foci. Kidneys, normal. Inguinal and cervical lymph nodes, swollen.	Liver, spleen, lung, lymph nodes.	*Br. abortus* (smooth).

The autopsy records with the isolations are presented in Table 8. From the guinea pigs receiving the living R organism and the killed S prototype, S strains were isolated from the liver, lung, spleen, and abscess at the point of injection. Smooth forms were also isolated from the guinea pigs receiving living R organisms alone; thus, both methods caused reversion of the R type to the S type equally well. However, the pigs receiving the killed S organism along with the living R organism showed more marked lesions and a more extensive diseased condition. In no instance was a rough form recovered.

STUDY OF REVERTED R STRAINS

The reverted R strains obtained by animal inoculation were isolated and studied to determine their behavior as measured by the typing methods used for classifying smooth strains of Brucella. It was found that all the strains had reverted to their original type, as measured by the hydrogen sulphide test and by their behavior on the thionin and basic fuchsin liver agar plates. All strains agglutinated the S immune serums.

DISCUSSION

The acceptance of methods of separating the Brucella group into species should not be considered as final until the effects of dissociation on such a grouping have been demonstrated.

Zdrodowski and his associates (*13*) show that any serological classification is wholly dependent on the phase of dissociation encountered. Marshall and Jared (*10*) found that the R type organisms failed to classify according to Huddleson on dye plates and hydrogen sulphide production. In the work presented in this paper, the R strains of Brucella failed to classify according to Huddleson's methods of grouping. However, the R forms of each species gave constant results. In Marshall and Jared's studies the species themselves varied on hydrogen sulphide production. The writers are of the opinion that pure R strains are characterized by a constancy of biochemical, cultural, and antigenic properties, and that the results reported by Marshall and Jared were due to the use of intermediate or partly rough types. This view is further sustained by their descriptions of the R types used. Their colonies were similar to those which the present writers designate as OS type and which appear in the early stages of impressed dissociation. This type of colony is the classic paramelitensis type. The mere fact that a change in colony appearance occurs, coupled with a change in antigenic behavior, does not argue that such a colony is a rough form. In this group of organism, as well as in some other groups of bacteria, antigenic and physiological changes occur before a change in colony appearance; hence these latter characters can not be taken as a sole measure of roughness, but rather as indications of progressive change. The fact that antigenic and physiological changes take place in the Brucella group as dissociation progresses from the S to the R state, and that these changes are rather gradual as measured by degree of transformation, and further that the colonial shift in structure follows the antigenic and physiologic changes makes any method of classifying this group unreliable, unless it is definitely known that the organisms in question are true S type. This is particularly true of any serological classification, especially as regards the agglutinin absorption method.

The constancy and reliability of Huddleson's dye plate and hydrogen sulphide production methods of classification, as regards S type organisms, is emphasized by this study. The fact that the R types, although having physiological and biochemical actions quite different from the S type, on reversion return to the same physiological and biochemical reactions, argues for a marked constancy of characters as represented by these tests. This work thus confirms that of Huddleson. The variability of the results of other workers using these tests may perhaps be explained on the dissociative type of organism tested.

SUMMARY

Rough forms of Brucella were obtained by aging on liver-infusion agar slants, followed by further aging in nutrient veal broth.

The rough forms of Brucella studied were similar to pure-line rough strains of the paratyphoid group.

Classification of S forms of Brucella by dye plates and hydrogen sulphide production does not hold for rough forms.

Rough forms of Brucella were changed to S type by passage through guinea pigs.

The value of typing S forms of Brucella by dye plates and hydrogen sulphide is confirmed by the return of R types to their original groups by these two methods.

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EFFECTS OF FORMALDEHYDE ON CERATOSTOMELLA FIMBRIATA AND THE SWEETPOTATO[1]

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INTRODUCTION

The objective in disinfecting sweetpotatoes *(Ipomoea batatas)* is to obtain with one treatment a practical control of all major seed-borne fungous parasites including *Monilochaetes infuscans* E. and H., *Fusarium batatatis* Woll., and *Ceratostomella fimbriata* (E. and H.) Elliot. This does not necessarily involve destroying these organisms when in the tissues, since diseased sweetpotatoes with the characteristic black spots are readily discarded by careful selection during the harvest and just before bedding. The destruction of spores, which are frequently abundant on the sweetpotato and germinate well in the plant bed, is of greater significance, since sweetpotatoes are sometimes contaminated with spores even when no symptoms of disease are discernible.

Mercuric chloride and formaldehyde disinfectants have been used for some time in treating sweetpotatoes immediately before bedding them to germinate new plants. Some growers claim to have obtained good control of sweetpotato diseases in general with both chemicals. Others have been disappointed, especially in the ineffectiveness of formaldehyde against *Monilochaetes infuscans*[2] and *Ceratostomella fimbriata*. Mercuric chloride is more widely recommended, but formaldehyde is suggested as a substitute by a number of investigators[3] as a general disinfectant of the sweetpotato. The suggestion that formaldehyde be employed as a substitute for mercuric chloride has resulted in its use in many localities where it seems to be more readily obtained than mercuric chloride. However, as a general disinfectant for the various fungi that attack the sweetpotato, preference is given to mercuric chloride.

If formaldehyde is used as a disinfectant, 1 pint, containing 40 per cent gas, should be used in 30 gallons of water, and it is suggested that the sweetpotatoes be treated for 5[3] or 15 minutes.[4] Treatment for a period longer than 15 minutes in a solution of formaldehyde of this strength has been found to be injurious.[4] The sweetpotatoes should be washed after this treatment.[3] The strength of the formaldehyde and the length of the treatment used in disinfecting will not control black rot, the most important seed-borne disease of sweetpotatoes.

It is the purpose of this paper to give the results of a study of the various dilutions of formaldehyde used and the length of treatment necessary to control the causal fungus of black rot and to discuss the injurious effects of the chemical on the sweetpotatoes.

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[1] Received for publication Apr. 26, 1932; issued March, 1933. Paper No. 59 of the Journal Series of the North Carolina Experiment Station.

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METHODS

The White Jersey, a sport strain of a prolific Jersey variety which produces small white sweetpotatoes, was used in this study. This variety frequently produces 15 to 30 small sweetpotatoes on each plant. They are light colored and are most desirable for determining readily the dark-brown spots produced by *Ceratostomella fimbriata*. These small sweetpotatoes were more readily handled in the experiment than larger ones would have been. All varieties of sweetpotatoes, however, are very susceptible to black rot.

Twenty-five bushels of sweetpotatoes were used in the tests. When both the sweetpotato and the fungus were tested for tolerance to the chemical, whole sweetpotatoes were used. When the tolerance of the fungus to the chemical was tested, and when the sweetpotato was exposed to the chemical less than one minute, cross sections approximately one-half inch in thickness were used.

The inoculum consisted mostly of hyaline conidia, but olive to brown conidia, ascospores, and mycelium were also present to a limited extent. Spores for the inoculations were produced in covered culture dishes on freshly cut cross sections of raw sweetpotatoes. The sections were cut and immersed immediately in a heavy spore suspension in water, drained well, and transferred to the dishes in liter amounts. The incubation period was four days, the minimum period for obtaining the maximum number of hyaline spores. In all the tests an extremely large amount of inoculum was used.

The inoculating medium was prepared by scraping spores from the sweetpotato sections and mixing them thoroughly with water. Equal quantities of the spore suspension were placed in containers of equal size, quart fruit jars or 1-gallon crocks. When whole sweetpotatoes were treated, 1-gallon and 4-gallon crocks were used which contained, respectively, one-half gallon and 2 gallons of water and both spores and formaldehyde in amounts depending on the length of treatment and the quantity of sweetpotatoes.

When the fungus and sweetpotatoes were treated simultaneously, the formaldehyde was added to the water, and after the solution had been stirred well, the sweetpotatoes were added. At the end of the designated period of treatment, they were removed and immediately rinsed with water. By this method some spores were removed but never enough to affect the results. The whole sweetpotatoes were incubated in glass-covered culture dishes for 10 days, but very accurate results were obtained by the eighth day. Through the glass covers it was possible to observe germination, which was as quickly obtained as when sweetpotatoes are bedded in sand, the practical method of securing new plants.

When the fungus was tested for tolerance to the chemical, freshly cut cross sections of the sweetpotatoes were used to check the result, since the small percentages of spores that germinated in the liquid proved an unsatisfactory means of interpreting the results. After the designated period of treatment, the chemical solution with the spores was poured over the sweetpotatoes and immediately returned to the original container. The sweetpotato sections were always rinsed in water to remove the formaldehyde.

An average temperature of 21° C. was used during the incubation period, since optimum growth of *Ceratostomella fimbriata* is produced

at that temperature.[5] The temperature during these tests varied between 20° and 22°. The relative humidity in the incubation containers was kept at about 100 per cent. Sterile containers were used in all tests. An occasional contamination of *Rhizopus nigricans* occurred, but only after the black-rot disease was well developed.

A commercially pure grade of formaldehyde containing 40 per cent gas was used in all the tests in this study, although in preliminary studies one containing 37 per cent gas was shown to be only slightly less effective.

Tap water from the city system was used. The reaction of this water varied between pH 7.5 and 9. This range favored the optimum growth of *Ceratostomella fimbriata*.

INJURIOUS EFFECTS OF FORMALDEHYDE AND WATER ON THE SWEETPOTATO

The treatment of cut sections and whole sweetpotatoes with formaldehyde was shown to have very injurious effects and also favored greater penetration of the tissues by *Ceratostomella fimbriata*. The cross sections of freshly cut tissues when washed thoroughly after treatment showed a tolerance of formaldehyde in strengths of 1–25 for 5 minutes, 1–50 for 10 minutes, 1–75 for 15 minutes, 1–100 for 20 minutes, and 1–200 for 25 minutes. Even the sections of sweetpotato treated in these dilutions for the periods of tolerance given were later found to be more susceptible to Trichoderma, Rhizopus, Fusaria, Penicillium, and other organisms than were other sections treated for the same period in water. The whole sweetpotatoes behaved in a similar manner, since the periderm tissue was not always sound even on those that appeared to be sound or at least free from prominent mechanical injury. Furthermore, the small adventitious or feed roots developing from the cambium seemed to indicate absorption of the chemical as quickly as did the freshly cut tissues of the cross sections. (Fig. 1.) After establishing their tolerance limits to the greater strengths, sweetpotatoes were immersed for 6, 9, and 17 hours in dilutions of formaldehyde in increments of 1 to 100 up to 1 to 1,000. Severe injury, resulting mostly from water damage, followed all treatments including those in which cross sections of sweetpotatoes were immersed in water, for checks. (Fig. 2.) Observations of sweetpotatoes in water-logged plant beds indicated that they are easily injured, especially before germination begins, but the injury that developed from soaking them for a few hours was not expected to be so pronounced. The sweetpotatoes germinated new plants, but these were frequently destroyed when the soft rots developed. (Fig. 3.) In 10 days after treatment some of the sweetpotatoes were soft and elastic in character but not decomposed. They gave a distinct acetic acid taste and smell. Others developed soft rots and were quickly decomposed.

As a result of the injury caused by immersing sweetpotatoes in water, they showed a distinct grayish to brown discoloration just beneath and including the periderm and finally the whole sweetpotato. (Fig. 2.) Those immersed 4, 6, 9, and 17 hours in water later germinated well, but the rapid development of Trichoderma,

[5] LAURITZEN, J. I. INFECTION AND TEMPERATURE RELATIONS OF BLACK ROT OF SWEETPOTATOES IN STORAGE. Jour. Agr. Research 33: 663–673, illus. 1926.

Rhizopus, Fusaria, and Penicillium on the injured ones destroyed both sweetpotato and young plant. (Fig. 4.)

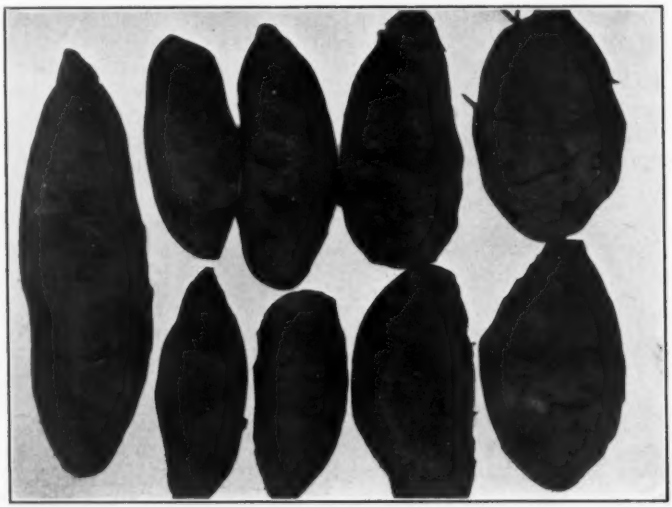

FIGURE 1.—Sweetpotatoes showing circular dehydrated spots resulting from formaldehyde injury

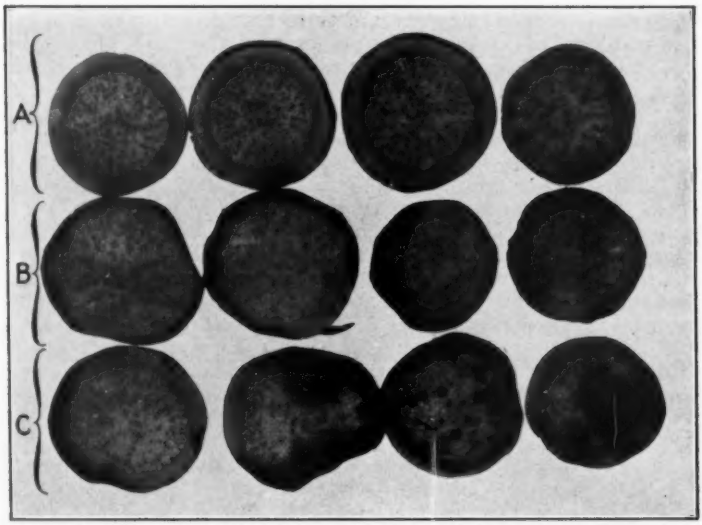

FIGURE 2.—Sections of sweetpotato: A, Healthy; B, showing breaking down, and browning and graying of tissues, especially beneath the periderm tissue, as a result of water injury; C, showing black-rot symptoms

The results of all tests clearly showed that the possibilities of a satisfactory use of formaldehyde for controlling *Ceratostomella fim-*

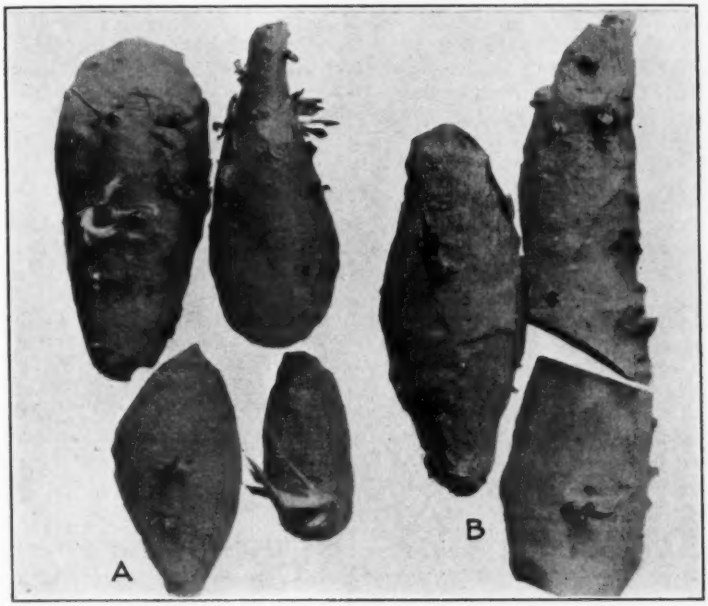

FIGURE 3.—Sweetpotatoes: A, Showing good germination after immersion in water for one minute; B, showing good germination, but with destruction of plants after the sweetpotato began to decay as a result of immersion in water for 17 hours

FIGURE 4.—Sweetpotatoes that were immersed in water for 17 hours and were later attacked and destroyed by: A, Trichoderma; B, Rhizopus; C, Fusaria; D, Penicillium

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briata must be based on short periods of immersion, possible control being established in the tolerance limits of the sweetpotato to the chemical, since extended treatments with the chemical at more dilute strengths resulted in greater susceptibility of the sweetpotato to various soft rot-producing fungi.

CONTROL OF CERATOSTOMELLA FIMBRIATA WITH VARIOUS DILUTIONS OF FORMALDEHYDE AFTER DIFFERENT PERIODS OF TREATMENT

When formaldehyde dilutions of 1–100, 1–300, 1–500, 1–700, 1–900, 1–1,000, 1–1,200, 1–1,500, and 1–2,000 were impregnated with a heavy inoculum of hyaline and brown conidia and ascospores of *Ceratostomella fimbriata* and incubated for five days at 20° to 22° C. there was a slight germination of the fungus in the 1–1,000 and more dilute solutions, but never enough germination for accurate estimation of the kill. Furthermore, there was no germination of spores in some solutions in which heavy infection was later obtained by transferring the spores to raw sweetpotatoes. At the end of 10 days the ungerminated spores, which showed no injury microscopically, were transferred to freshly cut cross sections of sweetpotatoes in sterile fruit jars and incubated for six days. The black-rot disease developed on sweetpotatoes inoculated with spores subjected to the 1–900 and 1–1,000 dilutions.

Formaldehyde dilutions were then prepared in liter quantities beginning with 1–50, 1–75, 1–100, 1–150, and 1–200 up to 1–1,100 and were used for treating the spores for various intervals. In order to handle these cultures on a uniform time basis for completing the inoculations at the designated period, spores were added to the separate cultures at 1-minute intervals. The spores were subjected to the formaldehyde for periods of ½, 1, 4, 6, 9, and 18 hours. Sweetpotatoes were then inoculated with spores that had been treated in the cultures for the various periods. The data given in Table 1 show the effectiveness of the treatments.

TABLE 1.—*Control of Ceratostomella fimbriata with various strengths of formaldehyde for different periods of time*[a]

Length of treatment (hours)	Length of time (days) when fungus was observed after treatment with formaldehyde at dilution indicated [a] [b]												
	1–50	1–75	1–100	1–150	1–200	1–250	1–300	1–350	1–400	1–450	1–500	1–550	1–600	Check
½	0	9	9	4	4	4	4	4	4	4	4	4	4	4
1	0	0	9	9	9	6	9	6	6	4	4	4	4	4
4	0	0	0	0	9	9	9	6	6	6	6	6	4	4
6	0	0	0	0	0	9	9	6	9	6	6	6	6	4
9	0	0	0	0	0	0	9	6	6	6	6	6	6	4
18	0	0	0	0	0	0	0	0	0	0	0	9	9	4

[a] 0 indicates that no disease developed. [b] 40 sweetpotato sections duplicated used in tests.

Black rot developed on the sweetpotatoes inoculated with spores taken from the formaldehyde dilutions of 1–550 to 1–1,100 after treatment for the various periods, which showed that the chemical at these strengths was not effective, even when the spores were treated 18 hours. Table 1, therefore, includes only the results obtained with dilutions of from 1–50 to 1–600 and the check. The number of days after inoculation required for the disease to develop on sweetpotatoes treated in the various dilutions for periods less than 18 hours, are

given. At the end of 12 days no disease had developed on sweetpotatoes inoculated with spores treated for one-half hour in the 1–50 dilution, for 1 hour in the 1–75 dilution, for 4 hours in the 1–100 and 1–150 dilutions, for 9 hours in the 1–250 dilution, and for 18 hours in dilutions of between 1–300 and 1–500. The "0" in this table indicates that no disease had developed at the end of the 12-day incubation period.

PERCENTAGES OF BLACK-ROT INFECTION OF SWEETPOTATOES DEVELOPING FROM SPORE INOCULUMS FROM 30 DILUTIONS OF DIFFERENT STRENGTHS AFTER DIFFERENT PERIODS OF TREATMENT

The percentages of infection given in Table 2 show the effects of the strengths of the chemical on the fungus, and the differences obtained in the infections. The results partly explain why the chemical at some of the more dilute strengths in a few instances gave less control than at those slightly stronger. There was no infection after treating the fungus one-half hour in dilutions of 1–25 and 1–50. Infections of 4.2, 13.5, and 14.2, and 100 per cent were obtained when sweetpotatoes were inoculated with spores treated for one-half hour in the 1–75, 1–100, 1–150, and 1–200 dilutions, respectively. Some spores lived in the 1–100, 1–150, and 1–200 dilutions 1 hour, and a few were not killed in the 1–200 dilution after an immersion of 4 hours. When spores were tested after treatment in the 1–250 dilution for ½, 1, 4, 6, and 9 hours, the infection was 100, 15.6, 6.6, 3.2, and 0 per cent, respectively. Results of a similar character in the gradual reduction of viable spores are shown for each of the numerous treatments, which show clearly that there is a marked variation in the resistance of the spores of this fungus to formaldehyde.

TABLE 2.—*Percentage of black-rot infection of sweetpotatoes developing from inoculations with spores that had been treated with various strengths of formaldehyde for different periods of time* [a]

Formaldehyde dilution used	Percentage infection of sweetpotatoes inoculated with spores treated with formaldehyde for the period indicated					
	½ hour	1 hour	4 hours	6 hours	9 hours	18 hours
1-25	0	0	0	0	0	0
1-50	0	0	0	0	0	0
1-75	4.2	0	0	0	0	0
1-100	13.5	2.5	0	0	0	0
1-150	14.2	3.8	0	0	0	0
1-200	100.0	.5	.3	0	0	0
1-250	100.0	15.6	6.6	3.2	0	0
1-300	100.0	25.0	7.5	6.2	4.1	0
1-350	100.0	36.1	4.7	2.6	0	0
1-400	100.0	28.3	7.8	3.1	0	0
1-450	100.0	100.0	5.9	3.7	4.1	0
1-500	100.0	100.0	13.1	5.9	3.0	0
1-550	100.0	100.0	15.5	6.6	5.9	2.0
1-600	100.0	100.0	23.6	11.5	8.3	2.3
1-650	100.0	100.0	13.0	4.7	4.7	2.4
1-700	100.0	100.0	12.4	12.5	8.6	4.3
1-750	100.0	100.0	20.0	17.9	9.3	3.5
1-800	100.0	100.0	20.0	20.0	5.0	3.2
1-850	100.0	100.0	20.0	6.6	4.1	3.3
1-900	100.0	100.0	7.3	4.5	4.0	3.1
1-950	100.0	100.0	13.1	7.1	5.3	3.1
1-1,000	100.0	100.0	23.0	16.6	12.0	10.0
1-1,100	100.0	100.0	40.0	22.5	15.4	7.5
1-1,200	100.0	100.0	100.0	31.8	20.2	3.6
1-1,300	100.0	100.0	100.0	16.6	13.6	7.6
1-1,500	100.0	100.0	100.0	28.9	100.0	8.3
Check	100.0	100.0	100.0	100.0	100.0	100.0

[a] 40 sweetpotato sections duplicated used in tests.

The results of treatments of all spores that developed on cut sweetpotatoes three and six days after inoculation indicated that the younger spores were less resistant to the chemical than were the older spores even of the same type. However, a more scientific explanation concerning this relation must await the development of a satisfactory means of separating spores of different ages. The treatment was repeated three times, with similar results. The effects were most manifest on the margins or near points where control was almost obtained but always clearly defined as the time of treatment was increased. On the margins control was sometimes obtained in weaker dilutions, possibly because of a difference in spore resistance. In this test an infection of 4.1 per cent of diseased sweetpotatoes was obtained after spores were taken from the 1–300 dilution after a treatment of 9 hours, but no disease developed after inoculating them with spores from the 1–350 and 1–400 dilutions when the treatment was also 9 hours. An average of 40 sweetpotato sections were used, and in most instances the low percentages shown here represent a single infection. It is, therefore, possible that the fact that weaker dilutions showed greater control for the same period of treatment than did a few slightly stronger ones may have been due to the sweetpotatoes failing to pick up the few spores that were not killed. Furthermore, the few viable spores that survived after the longer treatments may also have been removed during the rinsing. The check inoculations showed uniform infection after the spores had remained 18 hours in water. Six consecutive inoculations with the same inoculum, similar in amount to that used in the treatments, showed heavy infections of equal intensity in the checks.

EFFECTS OF FORMALDEHYDE IN VARIOUS STRENGTHS FOR DIFFERENT PERIODS OF TREATMENT ON CERATOSTOMELLA FIMBRIATA AND THE SWEETPOTATO

Formaldehyde solutions in strengths of 1–300, 1–400, 1–500, 1–600, 1–700, 1–800, 1–900, 1–1,000 and 1–1,100 impregnated with spores of *Ceratostomella fimbriata* were prepared. Sweetpotatoes were treated in these solutions for 6, 9, and 17 hours. The spores and sweetpotatoes, therefore, were treated simultaneously for similar periods.

Formaldehyde in 1–25, 1–50, 1–75, and 1–100 dilutions was used for treating cut pieces and whole sweetpotatoes. These treatments were based on the tolerance of the sweetpotato and the fungus to the chemical as shown in previous tests. The length of the treatments was both less and greater than that shown to give control in previous tests. The results of treating whole sweetpotatoes, with the exception of other shorter and longer treatments used, are given in Table 3. The disease did not develop on sweetpotatoes when the spores were treated 14 minutes in the 1–25 dilution. There was 9.1 per cent infection after the 12-minute treatment, and even after the 1-minute treatment some kill was obtained as indicated by less than 100 per cent infection. The spores treated for 35 minutes in the 1–50 dilution developed 8.2 per cent infection but not any after treatment for 40 minutes. Black rot developed on sweetpotatoes inoculated with spores treated for 65 minutes in the 1–75 dilution, but there was no infection from spores treated for 70 minutes. The 1–100 dilution killed the spores after treatment for 100 minutes, but 18.1 per cent infection developed from spores that had been treated 90 minutes.

The number of black rot spots developing on the sweetpotatoes inoculated with spores from the various solutions tended to increase with the greater dilutions of the chemical and with the shortening of the treatment from the point at which control was obtained. The check inoculation gave a maximum of 40 black-rot spots on sweetpotatoes. There were 7 spots on the sweetpotatoes after a 1-minute treatment of both fungus and sweetpotatoes in the 1–25 dilution, 3 spots after 5 minutes, and 1 spot after 8 minutes.

Injury resulted from the use of formaldehyde at all strengths when the treatment was given over a period of time sufficient to kill the spores and persisted even when the treated sweetpotatoes were washed well in running water.

TABLE 3.—*The effect on sweetpotatoes and the fungus Ceratostomella fimbriata of using formaldehyde of various strengths for different periods of time* [a]

Formaldehyde dilution used	Length of treatment	Infection produced	Maximum number of spots on potatoes	Injury to sweetpotatoes	Formaldehyde dilution used	Length of treatment	Infection produced	Maximum number of spots on potatoes	Injury to sweetpotatoes
	Minutes	*Per cent*				*Minutes*	*Per cent*		
1–25	5	66.0	3	Slight.	1–75	45	44.4	3	Severe.
	6	50.0	2	Do.		52	44.4	3	Do.
	8	10.0	1	Moderate.		55	55.5	3	Do.
	10	12.5	1	Severe.		60	13.3	1	Very severe.
	12	9.1	1	Do.		65	8.3	1	Do.
	14	0	0	Very severe.		70	0	0	Do.
1–50	10	100.0	4	Slight.	1–100	30	100.0	12	Slight.
	15	50.0	3	Do.		40	100.0	7	Do.
	20	60.0	2	Do.		52	100.0	7	Severe.
	25	11.1	2	Severe.		60	100.0	5	Do.
	30	12.5	2	Do.		70	60.0	2	Do.
	35	8.2	1	Very severe.		80	20.0	1	Do.
	40	0	0	Do.		90	18.1	1	Do.
1–75	30	100.0	6	Slight.		100	0	0	Very severe.
	35	41.7	4	Severe.	Check	.....	100.0	40	None.
	40	60.0	4	Do.					

[a] 40 whole sweetpotatoes in duplicate used in tests.

SUMMARY

A study of the effects of different strengths of formaldehyde used for various periods in treating the fungus *Ceratostomella fimbriata* (E. and H.) Elliot and the sweetpotato, shows that formaldehyde is not a practical or satisfactory disinfectant for sweetpotatoes and indicates that there is no safe strength and length of treatment that the chemical can be used for treating them.

The spores of *Ceratostomella fimbriata* were controlled in formaldehyde containing 40 per cent gas after 14 minutes in a strength of 1–25, 40 minutes in 1–50, 70 minutes in 1–75, 100 minutes in 1–100, 4 hours in 1–150, 6 hours in 1–200, and 9 hours in 1–250.

Some spores were killed even in weak dilutions and after short periods of exposure to the chemical, whereas others required a much longer treatment, indicating greater resistance for the same type of spore.

The sweetpotato was severely injured by the chemical in 1–25, 1–50, 1–75, 1–100, and 1–150 strengths even at short exposures that did not kill the spores. Treatments for four hours in 1–200, 1–250, and 1–300 dilutions did not control the fungus, and this length of treatment caused a definite breaking down of the sweetpotato and greater susceptibility to decay from other fungi.

Sweetpotatoes injured by both chemical and water were very susceptible to Trichoderma, Rhizopus, Penicillium, Fusaria, and other organisms which frequently destroyed the sweetpotato or suppressed the germination of new plants.

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